Document Title

# Sexually antagonistic mitonuclear coevolution in duplicate oxidative phosphorylation genes.

2019

Integrative and comparative biology

Havird, Justin C

McConie, Hunter J

Mitochondrial function is critical in eukaryotes. To maintain an adequate supply of energy, precise interactions must be maintained between nuclear- and mitochondrial-encoded gene products. Such interactions are paramount in chimeric enzymes such as the oxidative phosphorylation (OXPHOS) complexes. Mutualistic coevolution between the two genomes has therefore been suggested to be a critical, ubiquitous feature of eukaryotes that acts to maintain cellular function. However, mitochondrial genomes can also act selfishly and increase their own transmission at the expense of organismal function. For example, male-harming mutations are predisposed to accumulate in mitochondrial genomes due to their maternal inheritance ("mother's curse"). Here, we investigate sexually antagonistic mitonuclear coevolution in nuclear-encoded OXPHOS paralogs from mammals and Drosophila. These duplicate genes are highly divergent but must interact with the same set of mitochondrial-encoded genes. Many such paralogs show testis-specific expression, prompting previous hypotheses suggesting they may have evolved under selection to counteract male-harming mitochondrial mutations. We found increased rates of evolution in OXPHOS paralogs with testis-specific expression in mammals and Drosophila, supporting this hypothesis. However, further analyses suggested such patterns may be due to relaxed, not positive selection, especially in Drosophila. Structural data also suggest mitonuclear interactions do not play a major role in the evolution of many OXPHOS paralogs in a consistent way. In conclusion, no single OXPHOS paralog met all our criteria for being under selection to counteract male-harming mitochondrial mutations. We discuss alternative explanations for the drastic patterns of evolution in these genes, including, mutualistic mitonuclear coevolution, adaptive subfunctionalization after gene duplication, and relaxed selection on OXPHOS in male tissues.

# Inhibition of the chimeric DnaJ-PKAc enzyme by endogenous inhibitor proteins.

2019

Journal of cellular biochemistry

Averill, April M

Rehman, Hibba Tul

Charles, Joseph W

Dinh, Timothy A

Danyal, Karamatullah

Verschraegen, Claire F

Stein, Gary S

Dostmann, Wolfgang R

Ramsey, Jon E

The chimeric DnaJ-PKAc enzymeresulting from an approximately 400-kb deletion of chromosome 19 is a primary contributor to the oncogenic transformation that occurs in fibrolamellar hepatocellular carcinoma, also called fibrolamellar carcinoma (FLC). This oncogenic deletion juxtaposes exon 1 of the DNAJB1 heat shock protein gene with exon 2 of the PRKACA gene encoding the protein kinase A catalytic subunit, resulting in DnaJ-PKAc fusion under the transcriptional control of the DNAJB1 promoter. The expression of DnaJ-PKAc is approximately 10 times that of wild-type (wt) PKAc catalytic subunits, causing elevated and dysregulated kinase activity that contributes to oncogenic transformation. In normal cells, PKAc activity is regulated by a group of endogenous proteins, termed protein kinase inhibitors (PKI) that competitively inhibit PKAc and assist with the nuclear export of the enzyme. Currently, it is scarcely known whether interactions with PKI are perturbed in DnaJ-PKAc. In this report, we survey existing data sets to assess the expression levels of the various PKI isoforms that exist in humans to identify those that are candidates to encounter DnaJ-PKAc in both normal liver and FLC tumors. We then compare inhibition profiles of wtPKAc and DnaJ-PKAc against PKI and demonstrate that extensive structural homology in the active site clefts of the two enzymes confers similar kinase activities and inhibition by full-length PKI and PKI-derived peptides.

# Structural Determinants of Substrate Specificity of Omega-3 Desaturases from Mortierella alpina and Rhizophagus irregularis by Domain-Swapping and Molecular Docking.

2019

International journal of molecular sciences

Rong, Chunchi

Chen, Haiqin

Tang, Xin

Gu, Zhennan

Zhao, Jianxin

Zhang, Hao

Chen, Yongquan

Chen, Wei

Although various omega-3 fatty acid desaturases (omega3Des) have been identified and well-studied regarding substrate preference and regiospecificity, the molecular mechanism of their substrate specificities remains to be investigated. Here we compared two omega3Des, FADS15 from Mortierella alpina and oRiFADS17 from Rhizophagus irregularis, which possessed a substrate preference for linoleic acid and arachidonic acid, respectively. Their sequences were divided into six sections and a domain-swapping strategy was used to test the role of each section in catalytic activity. Heterologous expression and fatty acid experiments of hybrid enzymes in Saccharomyces cerevisiae INVSc1 indicated that the sequences between his-boxes I and II played critical roles in influencing substrate preference. Based on site-directed mutagenesis and molecular docking, the amino acid substitutions W129T and T144W, located in the upper part of the hydrocarbon chain, were found to be involved in substrate specificity, while V137T and V152T were confirmed to interfere with substrate recognition. This study provides significant insight into the structure-function relationship of omega3Des.

# Bioinformatic and functional evaluation of actinobacterial piperazate metabolism.

2019

ACS chemical biology

Hu, Yifei

Qi, Yunci

Stumpf, Spencer D

D'Alessandro, John M

Blodgett, Joshua A V

Piperazate (Piz) is a nonproteinogenic amino acid noted for its unusual N-N bond motif. Piz is a proline mimic that imparts conformational rigidity to peptides. Consequently, piperazyl molecules are often bioactive and desirable for therapeutic exploration. The in vitro characterization of Kutzneria enzymes KtzI and KtzT recently led to a biosynthetic pathway for Piz. However, Piz anabolism in vivo remained completely uncharacterized. Herein, we describe the systematic interrogation of actinobacterial Piz metabolism using a combination of bioinformatics, genetics, and select biochemistry. Following studies in Streptomyces flaveolus, Streptomyces lividans, and several environmental Streptomyces isolates, our data suggest that KtzI-type enzymes are conditionally dispensable for Piz production. We also demonstrate the feasibility of Piz monomer production using engineered actinobacteria for the first time. Finally, we show that some actinobacteria employ fused KtzI-KtzT chimeric enzymes to produce Piz. Our findings have implications for future piperazyl drug discovery, pathway engineering, and fine chemical bioproduction.

# IL-15-mediated reduction of mTORC1 activity preserves the stem cell memory phenotype of CAR-T cells and confers superior antitumor activity.

2019

Cancer immunology research

Alizadeh, Darya

Wong, Robyn A

Yang, Xin

Wang, Dongrui

Pecoraro, Joseph R

Kuo, Cheng-Fu

Aguilar, Brenda

Qi, Yue

Ann, David K

Starr, Renate

Urak, Ryan

Wang, Xiuli

Forman, Stephen J

Brown, Christine E

Efforts to improve the quality and fitness of chimeric antigen receptor (CAR)-engineered T cells, through CAR design or manufacturing optimizations, are critical to further enhance the potency of this promising therapy. A critical parameter influencing the effectiveness of CAR-T cell therapy is the T cell differentiation status of the final product, with less-differentiated, less-exhausted CAR-T cells being more therapeutically effective. In the current study, we demonstrate that CAR-T cells expanded in IL-15 alone (CAR-T/IL-15), even after extended ex vivo expansion, preserve a less-differentiated stem cell memory (Tscm) phenotype, defined as CD62L+ CD45RA+ CCR7+ as compared to cells cultured in IL-2 (CAR-T/IL-2). CAR-T/IL-15 cells exhibited reduced expression of exhaustion markers, higher anti-apoptotic properties, and increased proliferative capacity upon antigen challenge. Furthermore, CAR-T/IL-15 cells exhibited decreased mTORC1 activity, global reduction in expression of glycolytic enzymes and improved mitochondrial fitness. In fact, CAR-T/IL-2 cells cultured in rapamycin (mTORC1 inhibitor) shared similar phenotypic features as CAR-T/IL-15 suggesting that IL-15-mediated reduction of mTORC1 activity is responsible for preserving the Tscm phenotype. Importantly, CAR-T/IL-15 promoted superior antitumor responses in vivo in comparison to CAR-T/IL-2. Interestingly, inclusion of additional cytokines with IL-15, either IL-7 and/or IL-21, reduced the beneficial effects of IL-15 for CAR-T phenotype and antitumor potency. Taken together, our findings show that IL-15 preserves the Tscm phenotype by improving their metabolic fitness, and therefore has great potential for the future application of adoptive T cell therapy.

# EZH2 Inhibition in Ewing Sarcoma Upregulates GD2 Expression for Targeting with Gene-Modified T Cells.

2019

Molecular therapy : the journal of the American Society of Gene Therapy

Kailayangiri, Sareetha

Altvater, Bianca

Lesch, Stefanie

Balbach, Sebastian

Gottlich, Claudia

Kuhnemundt, Johanna

Mikesch, Jan-Henrik

Schelhaas, Sonja

Jamitzky, Silke

Meltzer, Jutta

Farwick, Nicole

Greune, Lea

Fluegge, Maike

Kerl, Kornelius

Lode, Holger N

Siebert, Nikolai

Muller, Ingo

Walles, Heike

Hartmann, Wolfgang

Rossig, Claudia

Chimeric antigen receptor (CAR) engineering of T cells allows one to specifically target tumor cells via cell surface antigens. A candidate target in Ewing sarcoma is the ganglioside GD2, but heterogeneic expression limits its value. Here we report that pharmacological inhibition of Enhancer of Zeste Homolog 2 (EZH2) at doses reducing H3K27 trimethylation, but not cell viability, selectively and reversibly induces GD2 surface expression in Ewing sarcoma cells. EZH2 in Ewing sarcoma cells directly binds to the promoter regions of genes encoding for two key enzymes of GD2 biosynthesis, and EZH2 inhibition enhances expression of these genes. GD2 surface expression in Ewing sarcoma cells is not associated with distinct in vitro proliferation, colony formation, chemosensitivity, or in vivo tumorigenicity. Moreover, disruption of GD2 synthesis by gene editing does not affect its in vitro behavior. EZH2 inhibitor treatment sensitizes Ewing sarcoma cells to effective cytolysis by GD2-specific CAR gene-modified T cells. In conclusion, we report a clinically applicable pharmacological approach for enhancing efficacy of adoptively transferred GD2-redirected T cells against Ewing sarcoma, by enabling recognition of tumor cells with low or negative target expression.

# Chimeric crRNAs with 19 DNA residues in the guide region show the retained DNA cleavage activity of Cas9 with potential to improve the specificity.

2019

Chemical communications (Cambridge, England)

Kim, Hyo Young

Kang, Seong Jae

Jeon, Yongmoon

An, Jinsu

Park, Jihyun

Lee, Hee Jae

Jang, Jeong-Eun

Ahn, JongSeong

Bang, Duhee

Chung, Hak Suk

Jeong, Cherlhyun

Ahn, Dae-Ro

We demonstrated that 19 out of 20 RNA residues in the guide region of crRNA can be replaced with DNA residues with high GC-contents. The cellular activity of the chimeric crRNAs to disrupt the target gene was comparable to that of the native crRNA.

# Structure of the zebrafish galectin-1-L2 and model of its interaction with the infectious hematopoietic necrosis virus (IHNV) envelope glycoprotein.

2019

Glycobiology

Ghosh, Anita

Banerjee, Aditi

Amzel, L Mario

Vasta, Gerardo R

Bianchet, Mario A

Galectins, highly conserved beta-galactoside-binding lectins, have diverse regulatory roles in development and immune homeostasis and can mediate protective functions during microbial infection. In recent years, the role of galectins in viral infection has generated considerable interest. Studies on highly pathogenic viruses have provided invaluable insight into the participation of galectins in various stages of viral infection, including attachment and entry. Detailed mechanistic and structural aspects of these processes remain undetermined. To address some of these gaps in knowledge, we used Zebrafish as a model system to examine the role of galectins in infection by infectious hematopoietic necrosis virus (IHNV), a rhabdovirus that is responsible for significant losses in both farmed and wild salmonid fish. Like other rhabdoviruses, IHNV is characterized by an envelope consisting of trimers of a glycoprotein that display multiple N-linked oligosaccharides and play an integral role in viral infection by mediating the virus attachment and fusion. Zebrafish's proto-typical galectin Drgal1-L2 and the chimeric-type galectin Drgal3-L1 interact directly with the glycosylated envelope of IHNV, and significantly reduce viral attachment. In this study, we report the structure of the complex of Drgal1-L2 with N-acetyl-D-lactosamine at 2.0 A resolution. To gain structural insight into the inhibitory effect of these galectins on IHNV attachment to the zebrafish epithelial cells, we modeled Drgal3-L1 based on human galectin-3, as well as, the ectodomain of the IHNV glycoprotein. These models suggest mechanisms for which the binding of these galectins to the IHNV glycoprotein hinders with different potencies the viral attachment required for infection.

# Expression of Active Staphylococcus aureus Tyrosine Kinases in a Human Cell Line.

2019

Biological & pharmaceutical bulletin

Fukazawa, Hidesuke

Fukuyama, Mari

Miyazaki, Yoshitsugu

Many bacteria encode tyrosine kinases that are structurally unrelated to their eukaryotic counterparts and are termed BY-kinases. Two BY-kinases, CapB1 and CapB2, have been identified in the Staphylococcus aureus genome. Although CapB1 and CapB2 share more than 70% homology, earlier studies with purified enzymes did not find any evident kinase activity in CapB1, whereas CapB2 was autophosphorylated on a C-terminal tyrosine cluster in the presence of the kinase modulator proteins CapA1 or CapA2. For the convenient analysis of BY-kinases, we attempted to express CapB2 in an active form in a mammalian cell line. To this end, the C-terminal activation domain of CapA1 was attached to the N-terminus of CapB2, and the resulting CapA1/CT-CapB2 chimera was further fused with various tags and transfected into HEK293T cells. Immunoblotting analyses showed that when fluorescent protein tags were attached to the N-terminus, CapA1/CT-CapB2 was both expressed and tyrosine phosphorylated in HEK293T cells. Mutation of the ATP-binding lysine abrogated tyrosine phosphorylation, indicating that tyrosine phosphorylation was catalyzed by the transfected bacterial kinase and not by endogenous cellular enzymes. Unexpectedly, mutation of the C-terminal tyrosine cluster did not abolish autophosphorylation. Further analyses revealed that CapA1/CT-CapB2 phosphorylated not only itself but also the attached fluorescent protein tag. Several domains and residues important for tyrosine kinase activity were identified from the production of various mutants. We also present data that CapB1, which was previously thought to be catalytically inert, may possess intrinsic kinase activity.

# Utility of Chimeric Mice with Humanized Liver for Predicting Human Pharmacokinetics in Drug Discovery: Comparison with in Vitro-in Vivo Extrapolation and Allometric Scaling.

2019

Biological & pharmaceutical bulletin

Naritomi, Yoichi

Sanoh, Seigo

Ohta, Shigeru

Predicting human pharmacokinetics (PK) such as clearance (CL) and volume of distribution (Vd) is a critical component of drug discovery. These predictions are mainly performed by in vitro-in vivo extrapolation (IVIVE) using human biological samples, such as hepatic microsomes and hepatocytes. However, some issues with this process have arisen, such as inconsistencies between in vitro and in vivo findings; the integration of predicted CYP, non-CYP and transporter-mediated human PK; and the difficulty of evaluating very metabolically stable compounds. Various approaches to solving these issues have been reported. Allometric scaling using experimental animals has also often been used. However, this method has also shown many problems due to interspecies differences, albeit that various correction methods have been proposed. Another approach involves the production of chimeric mice with humanized liver via the transplantation of human hepatocytes into mice. The livers of these mice are repopulated mostly with human hepatocytes and express human drug-metabolizing enzymes and drug transporters, suggesting that these mice are useful for solving the issues of IVIVE and allometric scaling, and more reliably predicting human PK. In this review, we summarize human PK prediction methods using IVIVE, allometric scaling and chimeric mice with humanized liver, and discuss the utility of predicting human PK in drug discovery by comparing these chimeric mice with IVIVE and allometric scaling.

# Transducing Protease Activity into DNA Output for Developing Smart Bionanosensors.

2019

Small (Weinheim an der Bergstrasse, Germany)

Bui, Hieu

Brown, Carl W 3rd

Buckhout-White, Susan

Diaz, Sebastian A

Stewart, Michael H

Susumu, Kimihiro

Oh, Eunkeu

Ancona, Mario G

Goldman, Ellen R

Medintz, Igor L

DNA can process information through sequence-based reorganization but cannot typically receive input information from most biological processes and translate that into DNA compatible language. Coupling DNA to a substrate responsive to biological events can address this limitation. A two-component sensor incorporating a chimeric peptide-DNA substrate is evaluated here as a protease-to-DNA signal convertor which transduces protease activity through DNA gates that discriminate between different input proteases. Acceptor dye-labeled peptide-DNAs are assembled onto semiconductor quantum dot (QD) donors as the input gate. Addition of trypsin or chymotrypsin cleaves their cognate peptide sequence altering the efficiency of Forster resonance energy transfer (FRET) with the QD and frees a DNA output which interacts with a tetrahedral output gate. Downstream output gate rearrangement results in FRET sensitization of a new acceptor dye. Following characterization of component assembly and optimization of individual steps, sensor ability to discriminate between the two proteases is confirmed along with effects from joint interactions where potential for cross-talk is highest. Processing multiple bits of information for a sensing outcome provides more confidence than relying on a single change especially for the discrimination between different targets. Coupling other substrates to DNA that respond similarly could help target other types of enzymes.

# Active immunization with norovirus P particle-based amyloid-beta chimeric protein vaccine induces high titers of anti-Abeta antibodies in mice.

2019

BMC immunology

Yang, Ping

Guo, Yongqing

Sun, Yao

Yu, Bin

Zhang, Haihong

Wu, Jiaxin

Yu, Xianghui

Wu, Hui

Kong, Wei

BACKGROUND: Active immunotherapy targeting amyloid-beta (Abeta) is a promising treatment for Alzheimer's disease (AD). Numerous preclinical studies and clinical trials demonstrated that a safe and effective AD vaccine should induce high titers of anti-Abeta antibodies while avoiding the activation of T cells specific to Abeta. RESULTS: An untagged Abeta1-6 chimeric protein vaccine against AD based on norovirus (NoV) P particle was expressed in Escherichia coli and obtained by sequential chromatography. Analysis of protein characteristics showed that the untagged Abeta1-6 chimeric protein expressed in soluble form exhibited the highest particle homogeneity, with highest purity and minimal host cell protein (HCP) and residual DNA content. Importantly, the untagged Abeta1-6 chimeric soluble protein could induce the strongest Abeta-specific humoral immune responses without activation of harmful Abeta-specific T cells in mice. CONCLUSIONS: The untagged Abeta1-6 chimeric protein vaccine is safe and highly immunogenic. Further research will determine the efficacy in cognitive improvement and disease progression delay.

# Potential Angiotensin Converting Enzyme Inhibitors from Moringa oleifera.

2019

Recent patents on biotechnology

Khan, Huma

Jaiswal, Varun

Kulshreshtha, Saurabh

Khan, Azhar

BACKGROUND: Hypertension is the chronic medical condition and it affect billions of people worldwide. Natural medicines are the main alternatives of treatment for a large majority of people suffering from hypertension. Niazicin-A, Niazimin-A, and Niaziminin-B compounds were reported from Moringa oleifera ethanolic leave extract having potential antihypertensive activity. OBJECTIVE: These compounds targeted with Angiotensin-converting enzyme [ACE] which is one of the main regulatory enzymes of the renin-angiotensin system. METHOD: Protein-ligand docking of these compounds with [ACE] [both domain N and C] was conceded out through Autodock vina and visualization was done by chimera. Pharmacokinetics study of these compounds was predicted by ADME-Toxicity Prediction Result: Niazicin-A, Niazimin-A, and Niaziminin-B showed high binding affinity with ACE and partially blocked the active sites of the enzyme. Niazicin-A, Niazimin-A and Niaziminin-B showed the estimated free binding energy of -7.6kcal/mol kcal/mol, -8.8kcal/mol and -8.0kcal/mol respectively with C-domain of ACE and -7.9kcal/mol, -8.5kcal/mol and -7.7kcal/mol respectively with N-domain of ACE. The compounds have shown better binding energy with angiotensin converting enzyme in comparison to Captopril -5.5kcal/mol and -5.6kcal/mol and Enalapril [standard] -8.4kcal/mol and -7.5kcal/mol with C and N domain respectively. CONCLUSION: Computationally, the selected bioactive molecules have shown better binding energy to known standard drugs which have been already known for inhibition of ACE and can further act as a pharmacophore for in vitro and in vivo studies in development of alternative medicine.

# Hematopoietic chimerism and donor-specific skin allograft tolerance after non-genotoxic CD117 antibody-drug-conjugate conditioning in MHC-mismatched allotransplantation.

2019

Nature communications

Li, Zhanzhuo

Czechowicz, Agnieszka

Scheck, Amelia

Rossi, Derrick J

Murphy, Philip M

Hematopoietic chimerism after allogeneic bone marrow transplantation may establish a state of donor antigen-specific tolerance. However, current allotransplantation protocols involve genotoxic conditioning which has harmful side-effects and predisposes to infection and cancer. Here we describe a non-genotoxic conditioning protocol for fully MHC-mismatched bone marrow allotransplantation in mice involving transient immunosuppression and selective depletion of recipient hematopoietic stem cells with a CD117-antibody-drug-conjugate (ADC). This protocol resulted in multilineage, high level (up to 50%), durable, donor-derived hematopoietic chimerism after transplantation of 20 million total bone marrow cells, compared with </= 2.1% hematopoietic chimerism from 50 million total bone marrow cells without conditioning. Moreover, long-term survival of bone marrow donor-type but not third party skin allografts is achieved in CD117-ADC-conditioned chimeric mice without chronic immunosuppression. The only observed adverse event is transient elevation of liver enzymes in the first week after conditioning. These results provide proof-of-principle for CD117-ADC as a non-genotoxic, highly-targeted conditioning agent in allotransplantation and tolerance protocols.

# Family hyperaldosteronism type I: a clinical case and review of literature.

2019

Terapevticheskii arkhiv

Chikladze, N M

Favorova, O O

Chazova, I E

Family hyperaldosteronism type I (glucocorticoids-remediable hyperaldosteronism) is a rare form of symptomatic arterial hypertension (AH), which often leads to the development of cerebrovascular complications. The disease is caused by the formation of the chimeric gene CYP11B2/CYP11B1. Expression of the chimeric gene is regulated by adrenocorticotropic hormone, and glucocorticoid therapy leads to a decrease in aldosterone secretion and normalization of blood pressure. The article presents the first clinical case of this monogenic disease diagnosed by us in Russia. The features of clinical course and treatment of the patient have been traced in the dynamics for 40 years of observation. Modern approaches to the diagnosis and treatment of this rare family form of hypertension are discussed.

# Ameliorating the Metabolic Burden of the Co-expression of Secreted Fungal Cellulases in a High Lipid-Accumulating Yarrowia lipolytica Strain by Medium C/N Ratio and a Chemical Chaperone.

2019

Frontiers in microbiology

Wei, Hui

Wang, Wei

Alper, Hal S

Xu, Qi

Knoshaug, Eric P

Van Wychen, Stefanie

Lin, Chien-Yuan

Luo, Yonghua

Decker, Stephen R

Himmel, Michael E

Zhang, Min

Yarrowia lipolytica, known to accumulate lipids intracellularly, lacks the cellulolytic enzymes needed to break down solid biomass directly. This study aimed to evaluate the potential metabolic burden of expressing core cellulolytic enzymes in an engineered high lipid-accumulating strain of Y. lipolytica. Three fungal cellulases, Talaromyces emersonii-Trichoderma reesei chimeric cellobiohydrolase I (chimeric-CBH I), T. reesei cellobiohydrolase II (CBH II), and T. reesei endoglucanase II (EG II) were expressed using three constitutive strong promoters as a single integrative expression block in a recently engineered lipid hyper-accumulating strain of Y. lipolytica (HA1). In yeast extract-peptone-dextrose (YPD) medium, the resulting cellulase co-expressing transformant YL165-1 had the chimeric-CBH I, CBH II, and EG II secretion titers being 26, 17, and 132 mg L(-1), respectively. Cellulase co-expression in YL165-1 in culture media with a moderate C/N ratio of approximately 4.5 unexpectedly resulted in a nearly two-fold reduction in cellular lipid accumulation compared to the parental control strain, a sign of cellular metabolic drain. Such metabolic drain was ameliorated when grown in media with a high C/N ratio of 59 having a higher glucose utilization rate that led to approximately twofold more cell mass and threefold more lipid production per liter culture compared to parental control strain, suggesting cross-talk between cellulase and lipid production, both of which involve the endoplasmic reticulum (ER). Most importantly, we found that the chemical chaperone, trimethylamine N-oxide dihydride increased glucose utilization, cell mass and total lipid titer in the transformants, suggesting further amelioration of the metabolic drain. This is the first study examining lipid production in cellulase-expressing Y. lipolytica strains under various C/N ratio media and with a chemical chaperone highlighting the metabolic complexity for developing robust, cellulolytic and lipogenic yeast strains.

# None

2019

Bulletin du cancer

Alcazer, Vincent

Delenda, Christophe

Poirot, Laurent

Depil, Stephane

DEVELOPMENT OF CAR T-CELLS IN SOLID TUMORS: CHALLENGES AND PERSPECTIVES: While Chimeric Antigen Receptor (CAR) T-cells have shown outstanding results in some hematologic malignancies, studies in solid tumors are less encouraging with poor response rates. Several factors can account for this lack of efficiency in solid tumors: heterogeneous expression or absence of specific target antigen (and so higher risk of toxicity), immunosuppressive microenvironment, homing and tumoral trafficking issues or lack of CAR T-cell persistence. Different approaches can be considered to overcome these resistance mechanisms: bispecific CARs, use of logic gates, combination with immune checkpoint inhibitors, engineered CAR T-cells resistant to immunosuppressive molecules, addition of chemokines or enzymes, combination with oncolytic virus, intra-tumoral administration, selection of memory T cell subpopulations and development of armored CAR T-cells secreting cytokines such as IL-12, -15 or -18. Last generation optimized CAR T-cell design should thus improve therapeutic efficiency. CAR-T cells may represent in a near future a therapeutic breakthrough also in solid tumors, especially in cold tumors and/or tumors lacking MHC class I expression. Cet article fait partie du numero supplement Les cellules CAR-T : une revolution therapeutique ? realise avec le soutien institutionnel des partenaires Gilead : Kite et Celgene.

# A novel cereblon modulator for targeted protein degradation.

2019

European journal of medicinal chemistry

Kim, Sung Ah

Go, Ara

Jo, Seung-Hyun

Park, Sun Jun

Jeon, Young Uk

Kim, Ji Eun

Lee, Heung Kyoung

Park, Chi Hoon

Lee, Chong-Ock

Park, Sung Goo

Kim, Pilho

Park, Byoung Chul

Cho, Sung Yun

Kim, Sunhong

Ha, Jae Du

Kim, Jeong-Hoon

Hwang, Jong Yeon

Immunomodulatory drugs (IMiDs) exert anti-myeloma activity by binding to the protein cereblon (CRBN) and subsequently degrading IKZF1/3. Recently, their ability to recruit E3 ubiquitin ligase has been used in the proteolysis targeting chimera (PROTAC) technology. Herein, we design and synthesize a novel IMiD analog TD-106 that induces the degradation of IKZF1/3 and inhibits the proliferation of multiple myeloma cells in vitro as well as in vivo. Moreover, we demonstrate that TD-428, which comprises TD-106 linked to a BET inhibitor, JQ1 efficiently induce BET protein degradation in the prostate cancer cell line 22Rv1. Consequently, cell proliferation is inhibited due to suppressed C-MYC transcription. These results, therefore, firmly suggest that the newly synthesized IMiD analog, TD-106, is a novel CRBN modulator that can be used for targeted protein degradation.

# Anaplastic lymphoma kinase fusions: Roles in cancer and therapeutic perspectives.

2019

Oncology letters

Cao, Zhifa

Gao, Qian

Fu, Meixian

Ni, Nan

Pei, Yuting

Ou, Wen-Bin

Receptor tyrosine kinase (RTK) anaplastic lymphoma kinase (ALK) serves a crucial role in brain development. ALK is located on the short arm of chromosome 2 (2p23) and exchange of chromosomal segments with other genes, including nucleophosmin (NPM), echinoderm microtubule-associated protein-like 4 (EML4) and Trk-fused gene (TFG), readily occurs. Such chromosomal translocation results in the formation of chimeric X-ALK fusion oncoproteins, which possess potential oncogenic functions due to constitutive activation of ALK kinase. These proteins contribute to the pathogenesis of various hematological malignancies and solid tumors, including lymphoma, lung cancer, inflammatory myofibroblastic tumors (IMTs), Spitz tumors, renal carcinoma, thyroid cancer, digestive tract cancer, breast cancer, leukemia and ovarian carcinoma. Targeting of ALK fusion oncoproteins exclusively, or in combination with ALK kinase inhibitors including crizotinib, is the most common therapeutic strategy. As is often the case for small-molecule tyrosine kinase inhibitors (TKIs), drug resistance eventually develops via an adaptive secondary mutation in the ALK fusion oncogene, or through engagement of alternative signaling mechanisms. The updated mechanisms of a variety of ALK fusions in tumorigenesis, proliferation and metastasis, in addition to targeted therapies are discussed below.

# Efficient oral vaccination by bioengineering virus-like particles with protozoan surface proteins.

2019

Nature communications

Serradell, Marianela C

Rupil, Lucia L

Martino, Roman A

Prucca, Cesar G

Carranza, Pedro G

Saura, Alicia

Fernandez, Elmer A

Gargantini, Pablo R

Tenaglia, Albano H

Petiti, Juan P

Tonelli, Renata R

Reinoso-Vizcaino, Nicolas

Echenique, Jose

Berod, Luciana

Piaggio, Eliane

Bellier, Bertrand

Sparwasser, Tim

Klatzmann, David

Lujan, Hugo D

Intestinal and free-living protozoa, such as Giardia lamblia, express a dense coat of variant-specific surface proteins (VSPs) on trophozoites that protects the parasite inside the host's intestine. Here we show that VSPs not only are resistant to proteolytic digestion and extreme pH and temperatures but also stimulate host innate immune responses in a TLR-4 dependent manner. We show that these properties can be exploited to both protect and adjuvant vaccine antigens for oral administration. Chimeric Virus-like Particles (VLPs) decorated with VSPs and expressing model surface antigens, such as influenza virus hemagglutinin (HA) and neuraminidase (NA), are protected from degradation and activate antigen presenting cells in vitro. Orally administered VSP-pseudotyped VLPs, but not plain VLPs, generate robust immune responses that protect mice from influenza infection and HA-expressing tumors. This versatile vaccine platform has the attributes to meet the ultimate challenge of generating safe, stable and efficient oral vaccines.

# Co-Expression of a Chimeric Protease Inhibitor Secreted by a Tumor-Targeted Salmonella Protects Therapeutic Proteins from Proteolytic Degradation.

2019

Journal of microbiology and biotechnology

Quintero, David

Carrafa, Jamie

Vincent, Lena

Lee, Hee Jong

Wohlschlegel, James

Bermudes, David

Sunflower trypsin inhibitor (SFTI) is a 14-amino-acid bicyclic peptide that contains a single internal disulfide bond. We initially constructed chimeras of SFTI with N-terminal secretion signals from the Escherichia coli OmpA and Pseudomonas aeruginosa ToxA, but only detected small amounts of protease inhibition resulting from these constructs. A substantially higher degree of protease inhibition was detected from a C-terminal SFTI fusion with E. coli YebF, which radiated more than a centimeter from an individual colony of E. coli using a culture-based inhibitor assay. Inhibitory activity was further improved in YebF-SFTI fusions by the addition of a trypsin cleavage signal immediately upstream of SFTI, and resulted in production of a 14-amino-acid, disulfide-bonded SFTI free in the culture supernatant. To assess the potential of the secreted SFTI to protect the ability of a cytotoxic protein to kill tumor cells, we utilized a tumor-selective form of the Pseudomonas ToxA (OTG-PE38K) alone and expressed as a polycistronic construct with YebF-SFTI in the tumor-targeted Salmonella VNP20009. When we assessed the ability of toxin-containing culture supernatants to kill MDA-MB-468 breast cancer cells, the untreated OTG-PE38K was able to eliminate all detectable tumor cells, while pretreatment with trypsin resulted in the complete loss of anticancer cytotoxicity. However, when OTG-PE38K was co-expressed with YebF-SFTI, cytotoxicity was completely retained in the presence of trypsin. These data demonstrate SFTI chimeras are secreted in a functional form and that co-expression of protease inhibitors with therapeutic proteins by tumor-targeted bacteria has the potential to enhance the activity of therapeutic proteins by suppressing their degradation within a proteolytic environment.

# Fine Tuning of Functional Features of the CuA Site by Loop-Directed Mutagenesis.

2019

Inorganic chemistry

Zitare, Ulises A

Szuster, Jonathan

Santalla, Maria C

Llases, Maria E

Morgada, Marcos N

Vila, Alejandro J

Murgida, Daniel H

Here we report the spectroscopic and electrochemical characterization of three novel chimeric CuA proteins in which either one or the three loops surrounding the metal ions in the Thermus thermophilus protein have been replaced by homologous human and plant sequences while preserving the set of coordinating amino acids. These conservative modifications mimic basic differences between CuA sites from different organisms and allow for fine tuning the energy gap between alternative electronic ground states of CuA.. This results in a systematic modulation of thermodynamic and kinetic electron transfer (ET) parameters and in the selection of one of two possible redox-active molecular orbitals, which differ in the ET reorganization energy by a factor of 2. Moreover, the ET mechanism is found to be frictionally controlled, and the modifications introduced into the different chimeras do not affect the frictional activation parameter.

# Amplification-free and direct fluorometric determination of telomerase activity in cell lysates using chimeric DNA-templated silver nanoclusters.

2019

Mikrochimica acta

Lee, Shi Ting

Rahman, Ruman

Muthoosamy, Kasturi

Mohamed, Nur Aliana Hidayah

Su, XiaoDi

Tayyab, Saad

New, Siu Yee

A fluorogenic probe has been developed for determination of telomerase activity using chimeric DNA-templated silver nanoclusters (AgNCs). The formation of AgNCs was investigated before (route A) and after (route B) telomerase elongation reaction. Both routes caused selective quenching of the yellow emission of the AgNCs (best measured at excitation/emission wavelength of 470/557 nm) in telomerase-positive samples. The quenching mechanism was studied using synthetically elongated DNA to mimic the telomerase-catalyzed elongation. The findings show that quenching is due to the formation of parallel G-quadruplexes with a -TTA- loop in the telomerase elongated products. The assay was validated using different cancer cell extracts, with intra- and interassay coefficients of variations of <9.8%. The limits of detection for MCF7, RPMI 2650 and HT29 cell lines are 15, 22 and 39 cells/muL. This represents a distinct improvement over the existing telomeric repeat amplification protocol (TRAP) assay in terms of time, sensitivity and cost. Graphical Abstract A method was developed using chimeric DNA-templated silver nanoclusters to detect telomerase activity directly in cell extracts. The sensitivity of this new method outperforms the traditional TRAP assay, and without the need for amplification.

# Acute myeloid leukemia with t(10;11)(p11-12;q23.3): Results of Russian Pediatric AML registration study.

2019

International journal of laboratory hematology

Zerkalenkova, Elena

Lebedeva, Svetlana

Kazakova, Anna

Tsaur, Grigory

Starichkova, Yulia

Timofeeva, Natalia

Soldatkina, Olga

Aprelova, Evgenia

Popov, Aleksandr

Ponomareva, Natalia

Baidun, Ludmila

Meyer, Claus

Novichkova, Galina

Maschan, Michael

Maschan, Aleksey

Marschalek, Rolf

Olshanskaya, Yulia

INTRODUCTION: Translocations involving the KMT2A gene (also known as MLL) are frequently diagnosed in pediatric acute leukemia cases with either lymphoblastic or myeloid origin. KMT2A is translocated to multiple partner genes, including MLLT10/AF10 localizing at chromosomal band 10p12. KMT2A-MLLT10 is one of the common chimeric genes diagnosed in acute leukemia with KMT2A rearrangement (8%), especially in acute myeloid leukemia (AML; 18%). MLLT10 is localized in very close proximity to two other KMT2A partner genes at 10p11-12-NEBL and ABI1, so they could not be distinguished by conventional cytogenetics. METHODS: In this work, we present a cohort of 28 patients enrolled into Russian Pediatric AML registration study carrying rearrangements between chromosomal regions 11q23.3 and 10p11-12. G-banding, FISH, reverse transcription PCR, and long-distance inverse PCR were used to characterize the KMT2A gene rearrangements in these patients. RESULTS: We demonstrate that 25 patients harbor the KMT2A-MLLT10 rearrangement, while three patients show the rare KMT2A rearrangements (2x KMT2A-NEBL; 1x KMT2A-ABI1). CONCLUSIONS: Therefore, the combination of cytogenetic and molecular genetic methods is of high importance in diagnosing cases with t(10;11)(p11-12;q23.3).

# The PROTAC technology in drug development.

2019

Cell biochemistry and function

Zou, Yutian

Ma, Danhui

Wang, Yinyin

Currently, a new technology termed PROTAC, proteolysis targeting chimera, has been developed for inducing the protein degradation by a targeting molecule. This technology takes advantage of a moiety of targeted protein and a moiety of recognizing E3 ubiquitin ligase and produces a hybrid molecule to specifically knock down a targeted protein. During the first decade, three pedigreed groups worked on the development of this technology. To date, this technology has been extended by different groups, aiming to develop new drugs against different diseases including cancers. This review summarizes the contributions of the groups for the development of PROTAC. SIGNIFICANCE OF THE STUDY: This review summarized the development of the PROTAC technology for readers and also presented the author's opinions on the application of the technology in tumor therapy.

# A sequentially responsive and structure-transformable nanoparticle with a comprehensively improved 'CAPIR cascade' for enhanced antitumor effect.

2019

Nanoscale

Xu, Chenfeng

Sun, Yu

Yu, Yulin

Hu, Mei

Yang, Conglian

Zhang, Zhiping

An intravenously administered drug delivery system should undergo a five-step 'CAPIR' cascade (circulation, accumulation, penetration, internalization and release), and the maximal efficiency of each step is of great importance to obtain the improved final therapeutic benefits and overall survival rate. Here, a pH/matrix metalloproteinase-9 (MMP9) sequentially responsive and continuously structure-transformable nanoparticle assembled from a doxorubicin (DOX)-conjugated peptide was exploited for comprehensively improving the 'CAPIR cascade' and eventually enhancing the therapeutic efficacy. The chimeric peptide can self-assemble into spherical nanoparticles (RGD-sNPs) at pH 7.4 with a particle size of 45.7 +/- 5.4 nm. By a combination of passive and active targeting mechanisms, RGD-sNPs achieved efficient accumulation at the tumor site ( approximately 15.1% ID g-1 within 24 h). Both in vitro and in vivo experiments revealed that RGD-sNPs can be transformed into rod-like nanoparticles (S-NFs) triggered by MMP9 that overexpressed in the tumor microenvironment, demonstrating remarkable advantages of deep tumor penetration, prolonged drug retention with approximately 3.7% ID g-1 at 96 h, and 2-fold enhanced internalization. Subsequently, S-NFs would respond to the intracellular weakly acidic stimuli to rapidly release DOX for induction of cytotoxicity and apoptosis. Meanwhile, the remaining peptide was further converted into long fibers (length >5 mum) with significant cytotoxicity, thereby exerting a synergistic antitumor effect. Thus RGD-sNPs displayed superior antitumor efficacy and extended the median survival period to 55 days. This provides a new horizon for the exploration of high-performance antitumor nanomedicines.

# Hemagglutinin-Neuraminidase and fusion genes are determinants of NDV thermostability.

2018

Veterinary microbiology

Liu, Tong

Song, Yang

Yang, Yanling

Bu, Yawen

Cheng, Jinlong

Zhang, Guozhong

Xue, Jia

Newcastle disease (ND) caused by infections with virulent strains of Newcastle disease virus (NDV) continues to be a threat for poultry industry worldwide. The prospect of developing a thermostable and effective NDV vaccine is still highly desirable. To investigate the determinants of thermostability in NDV, we generated recombinant NDV strains by exchanging viral hemagglutinin-neuraminidase (HN) gene or by mutating the fusion (F) gene. The results showed that the HN and F protein were both determinants of NDV thermostability. With increased thermostability, the HN protein-chimeric virus showed significantly reduced neuraminidase and hemadsorption activities, but its hemolytic activity was retained. We also found that changing the amino acid in the F protein cleavage sites, affected the thermostability as well as the pathogenicity and fusogenic capacity of the virus. Taken together, our results suggest that HN and F proteins both contribute to the thermostability of NDV, and other viral biological activities change as the thermostability of the virus changes. These findings should be of benefit to the development of a thermostable and efficacious NDV vaccine.

# Targeted Modification of the Cationic Anticancer Peptide HPRP-A1 with iRGD To Improve Specificity, Penetration, and Tumor-Tissue Accumulation.

2018

Molecular pharmaceutics

Hu, Cuihua

Huang, Yibing

Chen, Yuxin

The chimeric peptide HPRP-A1-iRGD, composed of a chemically conjugated tumor-homing/penetration domain (iRGD) and a cationic anticancer peptide domain (HPRP-A1), was used to study the effect of targeted modification to enhance the peptide's specificity, penetration, and tumor accumulation ability. The iRGD domain exhibits tumor-targeting and tumor-penetrating activities by specifically binding to the neuropilin-1 receptor. Acting as a homing/penetration domain, iRGD contributed to enhancing the tumor selectivity, permeability, and targeting of HPRP-A1 by targeted receptor dependence. As the anticancer active domain, HPRP-A1 kills cancer cells by disrupting the cell membrane and inducing apoptosis. The in vitro membrane selectivity toward cancer cells, such as A549 and MDA-MB-23, and human umbilical vein endothelial cells (HUVECs), normal cells, the penetrability assessment in the A549 3D multiple cell sphere model, and the in vivo tumor-tissue accumulation test in the A549 xenograft model indicated that HPRP-A1-iRGD exhibited significant increases in the selectivity toward membranes that highly express NRP-1, the penetration distance in 3D multiple cell spheres, and the accumulation in tumor tissues after intravenous injection, compared with HPRP-A1 alone. The mechanism of the enhanced targeting ability of HPRP-A1-iRGD was demonstrated by the pull-down assay and biolayer interferometry test, which indicated that the chimeric peptide could specifically bind to the neuropilin-1 protein with high affinity. We believe that chemical conjugation with iRGD to increase the specificity, penetration, and tumor-tissue accumulation of HPRP-A1 is an effective and promising approach for the targeted modification of peptides as anticancer therapeutics.

# Development of bisphenol A (BPA)-sensing indicator Arabidopsis thaliana which synthesizes anthocyanin in response to BPA in leaves.

2018

Ecotoxicology and environmental safety

Kim, DongGwan

Bahmani, Ramin

Ko, Jae-Heung

Hwang, Seongbin

Bisphenol A (BPA) is an estrogenic endocrine disruptor which disturbs a normal animal development. We generated an indicator plant that senses and provides a clear visual indicator of an estrogen-like compound BPA in the environment. We developed transgenic Arabidopsis lines expressing a construct designed to synthesize anthocyanin (thus showing a red color) in response to BPA. We transformed Arabidopsis with a recombinant vector containing the chimeric estrogen receptor (XVE region), LAP and coding region of PtrMYB119 (transcription factor involved in anthocyanin biosynthesis in poplar and Arabidopsis). Upon binding of the estrogen compound to the ligand-binding domain of E (estrogen receptor) in XVE, the XV domain binds to LAP promoter and triggering the transcription of PtrMYB119 with a subsequent enhancement of anthocyanin biosynthetic gene expression, resulting in anthocyanin synthesis. The leaves of the transgenic Arabidopsis line XVE-PtrMYB119 turned red in the presence of 10ppm BPA. The transcript level of PtrMYB119 peaked at day 3 of BPA exposure, then decreased to its minimal level at day 5. Similar expression patterns to that of PtrMYB119 were detected for genes encoding the anthocyanin biosynthetic enzymes chalcone synthase, chalcone flavanone isomerase, flavanone 3-hydroxylase, dihydroflavonol 4-reductase, anthocyanidin synthase, and UFGT (UGT78D2). The leaves of transgenic plants did not turn red in response to BPA at concentrations below 10ppm, but PtrMYB119 expression was induced by BPA at concentrations as low as 1 ppt BPA. Since this transgenic plant turns red in the presence of BPA without any experimental procedures, this line can be easily used by non-scientists.

# Galanin/GalR1-3 system: A promising therapeutic target for myocardial ischemia/reperfusion injury.

2018

Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie

Palkeeva, Marina

Studneva, Irina

Molokoedov, Alexander

Serebryakova, Larisa

Veselova, Oksana

Ovchinnikov, Michael

Sidorova, Maria

Pisarenko, Oleg

N-terminal fragments of galanin (2-11) and (2-15) are critical for binding to GalR1-3 receptors, members of the G-protein-coupled receptor superfamily, and are involved in myocardial protection against ischemia/reperfusion (I/R) injury. This study was designed to synthesize novel GalR1-3 agonists with improved properties and evaluate their efficiency as cardioprotective agents. Peptide agonists were synthesized by the automatic solid phase method using Fmoc technology and purified by preparative HPLC. Their chemical structure was identified by (1)H-NMR spectroscopy and MALDI-TOF mass spectrometry. Novel ligands of galanin receptors have greater solubility in water than natural galanin fragments. Cardiac function indices, myocardial infarct size and plasma activity of creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) were measured to assess the peptide bioactivity. Infusion of optimal concentrations of the peptides (210-240 muM) after global ischemia enhanced functional recovery of isolated rat heart during reperfusion. Intravenous administration of the peptides in a dose range of 1-2 mg/kg at the onset of reperfusion significantly reduced infarct size and plasma levels of CK-MB and LDH in rats in vivo. The chimeric ligand [betaAla14, His15]-galanin (2-15) exhibited the most beneficial effect on both models of I/R injury. The results suggest that pharmacological agonists of GalR1-3 receptors can be a rational basis for drug developments in the field of cardiovascular diseases.

# Treating osteosarcoma with CAR T cells.

2018

Scandinavian journal of immunology

Koksal, Hakan

Muller, Elisabeth

Inderberg, Else Marit

Bruland, Oyvind

Walchli, Sebastien

Novel therapies to treat patients with solid cancers that have developed resistance to chemotherapy represent unmet needs of considerable dimensions. In the present review, we will address the attempts to develop chimeric antigen receptor (CAR) targeted immunotherapy against osteosarcoma (OS). This aggressive cancer displays its peak incidence in children and young adults. The main cause of patient death is lung metastases with a 5-year survival as low as 5%-10% in the primary metastatic setting and 30% in the relapse situation, respectively. Effective adjuvant combination chemotherapy introduced more than 40 years ago improved the survival rates from below 20% to around 60% in patients; however, since then, no major breakthroughs have been made. The use of immune checkpoint inhibitors has been disappointing in OS, while other types of immunotherapies such as CAR T cells remain largely unexplored. Indeed, for CAR T-cell therapy to be efficacious, two main criteria need to be fulfilled: (a) CAR T cells should target an epitope selectively expressed on the cell surface of OS in order to prevent toxicities in normal tissues and (b) the target should also be widely expressed on OS metastases. These challenges have already been undertaken in OS and illustrate the difficulties in developing tomorrow's CAR-T treatment in a solid tumour. We will discuss the experiences with CAR-T therapy development and efficacy to combat the clinical challenges in OS.

# Acceptor Stem Differences Contribute to Species-Specific Use of Yeast and Human tRNA(Ser).

2018

Genes

Berg, Matthew D

Genereaux, Julie

Zhu, Yanrui

Mian, Safee

Gloor, Gregory B

Brandl, Christopher J

The molecular mechanisms of translation are highly conserved in all organisms indicative of a single evolutionary origin. This includes the molecular interactions of tRNAs with their cognate aminoacyl-tRNA synthetase, which must be precise to ensure the specificity of the process. For many tRNAs, the anticodon is a major component of the specificity. This is not the case for the aminoacylation of alanine and serine to their cognate tRNAs. Rather, aminoacylation relies on other features of the tRNA. For tRNA(Ser), a key specificity feature is the variable arm, which is positioned between the anticodon arm and the T-arm. The variable arm is conserved from yeast to human. This work was initiated to determine if the structure/function of tRNA(Ser) has been conserved from Saccharomyces cerevisiae to human. We did this by detecting mistranslation in yeast cells with tRNA(Ser) derivatives having the UGA anticodon converted to UGG for proline. Despite being nearly identical in everything except the acceptor stem, human tRNA(Ser) is less active than yeast tRNA(Ser). A chimeric tRNA with the human acceptor stem and other sequences from the yeast molecule acts similarly to the human tRNA(Ser). The 3:70 base pair in the acceptor stem (C:G in yeast and A:U in humans) is a prime determinant of the specificity. Consistent with the functional difference of yeast and human tRNA(Ser) resulting from subtle changes in the specificity of their respective SerRS enzymes, the functionality of the human and chimeric tRNA(Ser)UGG molecules was enhanced when human SerRS was introduced into yeast. Residues in motif 2 of the aminoacylation domain of SerRS likely participated in the species-specific differences. Trp290 in yeast SerRS (Arg313 in humans) found in motif 2 is proximal to base 70 in models of the tRNA-synthetase interaction. Altering this motif 2 sequence of hSerRS to the yeast sequence decreases the activity of the human enzyme with human tRNA(Ser), supporting the coadaptation of motif 2 loop(-)acceptor stem interactions.

# The application of prostate specific membrane antigen in CARTcell therapy for treatment of prostate carcinoma (Review).

2018

Oncology reports

Ullah, Kifayat

Addai Peprah, Frank

Yu, Feng

Shi, Haifeng

Adoptive cell transfer (ACT) is an emerging immunotherapy technique that restricts tumor growth and invasion in cancer patients. Among the different types of ACT, chimeric antigen receptor (CAR)Tcell therapy is considered to be the most advanced and a potentially powerful technique for the treatment of cancer in clinical trials. The primary aim of CARTcell therapy is to destroy cancer cells and therefore, it serves an important role in tumor immunotherapy. CARTcell therapy has been demonstrated to mainly treat blood cancer by targeting cluster of differentiation (CD)19, CD20, CD22, CD33 and CD123. However, the use of CARTcell therapy for treating solid tumors is currently under extensive investigation. With respect to prostate cancer, prostatic acid phosphatase, prostatespecific antigen, prostatespecific membrane antigen (PSMA), prostate stem cell antigen, Tcell receptor gamma alternate reading frame protein, transient receptor potentialp8 and sixtransmembrane epithelial antigen of the prostate 1 are among the identified target antigens for prostate tumors. However, mesothelin, fibroblast activation protein, epidermal growth factor receptor, carcinoembryonic antigen, disialoganglioside2 and human epidermal growth factor 2 are among the main targets of CARTcell therapy in the case of other types of solid tumors. The main challenges in CARTcell therapy are the selection of the target antigens and the modulation of the ideal tumor microenvironment for Tcells to fight against the cancer. The present review focuses on the 1st, 2nd, 3rd and 4th generations of antiPSMA CARs and their application for combating prostate carcinoma.

# RNA-mediated gene fusion in mammalian cells.

2018

Proceedings of the National Academy of Sciences of the United States of America

Gupta, Sachin Kumar

Luo, Liming

Yen, Laising

One of the hallmarks of cancer is the formation of oncogenic fusion genes as a result of chromosomal translocations. Fusion genes are presumed to form before fusion RNA expression. However, studies have reported the presence of fusion RNAs in individuals who were negative for chromosomal translocations. These observations give rise to "the cart before the horse" hypothesis, in which the genesis of a fusion RNA precedes the fusion gene. The fusion RNA then guides the genomic rearrangements that ultimately result in a gene fusion. However, RNA-mediated genomic rearrangements in mammalian cells have never been demonstrated. Here we provide evidence that expression of a chimeric RNA drives formation of a specified gene fusion via genomic rearrangement in mammalian cells. The process is: (i) specified by the sequence of chimeric RNA involved, (ii) facilitated by physiological hormone levels, (iii) permissible regardless of intrachromosomal (TMPRSS2-ERG) or interchromosomal (TMPRSS2-ETV1) fusion, and (iv) can occur in normal cells before malignant transformation. We demonstrate that, contrary to "the cart before the horse" model, it is the antisense rather than sense chimeric RNAs that effectively drive gene fusion, and that this disparity can be explained by transcriptional conflict. Furthermore, we identified an endogenous RNA AZI1 that functions as the "initiator" RNA to induce TMPRSS2-ERG fusion. RNA-driven gene fusion demonstrated in this report provides important insight in early disease mechanisms, and could have fundamental implications in the biology of mammalian genome stability, as well as gene-editing technology via mechanisms native to mammalian cells.

# Homologous recombination changes the context of Cytochrome b transcription in the mitochondrial genome of Silene vulgaris KRA.

2018

BMC genomics

Storchova, Helena

Stone, James D

Sloan, Daniel B

Abeyawardana, Oushadee A J

Muller, Karel

Walterova, Jana

Pazoutova, Marie

BACKGROUND: Silene vulgaris (bladder campion) is a gynodioecious species existing as two genders - male-sterile females and hermaphrodites. Cytoplasmic male sterility (CMS) is generally encoded by mitochondrial genes, which interact with nuclear fertility restorer genes. Mitochondrial genomes of this species vary in DNA sequence, gene order and gene content. Multiple CMS genes are expected to exist in S. vulgaris, but little is known about their molecular identity. RESULTS: We assembled the complete mitochondrial genome from the haplotype KRA of S. vulgaris. It consists of five chromosomes, two of which recombine with each other. Two small non-recombining chromosomes exist in linear, supercoiled and relaxed circle forms. We compared the mitochondrial transcriptomes from females and hermaphrodites and confirmed the differentially expressed chimeric gene bobt as the strongest CMS candidate gene in S. vulgaris KRA. The chimeric gene bobt is co-transcribed with the Cytochrome b (cob) gene in some genomic configurations. The co-transcription of a CMS factor with an essential gene may constrain transcription inhibition as a mechanism for fertility restoration because of the need to maintain appropriate production of the necessary protein. Homologous recombination places the gene cob outside the control of bobt, which allows for the suppression of the CMS gene by the fertility restorer genes. We found the loss of three editing sites in the KRA mitochondrial genome and identified four sites with highly distinct editing rates between KRA and another S. vulgaris haplotypes (KOV). Three of these highly differentially edited sites were located in the transport membrane protein B (mttB) gene. They resulted in differences in MttB protein sequences between haplotypes. CONCLUSIONS: Frequent homologous recombination events that are widespread in plant mitochondrial genomes may change chromosomal configurations and also the control of gene transcription including CMS gene expression. Posttranscriptional processes, e.g. RNA editing shall be evaluated in evolutionary and co-evolutionary studies of mitochondrial genes, because they may change protein composition despite the sequence identity of the respective genes. The investigation of natural populations of wild species such as S. vulgaris are necessary to reveal important aspects of CMS missed in domesticated crops, the traditional focus of the CMS studies.

# The phase separation underlying the pyrenoid-based microalgal Rubisco supercharger.

2018

Nature communications

Wunder, Tobias

Cheng, Steven Le Hung

Lai, Soak-Kuan

Li, Hoi-Yeung

Mueller-Cajar, Oliver

The slow and promiscuous properties of the CO2-fixing enzyme Rubisco constrain photosynthetic efficiency and have prompted the evolution of powerful CO2 concentrating mechanisms (CCMs). In eukaryotic microalgae a key strategy involves sequestration of the enzyme in the pyrenoid, a liquid non-membranous compartment of the chloroplast stroma. Here we show using pure components that two proteins, Rubisco and the linker protein Essential Pyrenoid Component 1 (EPYC1), are both necessary and sufficient to phase separate and form liquid droplets. The phase-separated Rubisco is functional. Droplet composition is dynamic and components rapidly exchange with the bulk solution. Heterologous and chimeric Rubiscos exhibit variability in their tendency to demix with EPYC1. The ability to dissect aspects of pyrenoid biochemistry in vitro will permit us to inform and guide synthetic biology ambitions aiming to engineer microalgal CCMs into crop plants.

# SUMOylation of PCNA by PIAS1 and PIAS4 promotes template switch in the chicken and human B cell lines.

2018

Proceedings of the National Academy of Sciences of the United States of America

Mohiuddin, Mohiuddin

Evans, Terry John

Rahman, Md Maminur

Keka, Islam Shamima

Tsuda, Masataka

Sasanuma, Hiroyuki

Takeda, Shunichi

DNA damage tolerance (DDT) releases replication blockage caused by damaged nucleotides on template strands employing two alternative pathways, error-prone translesion DNA synthesis (TLS) and error-free template switch (TS). Lys164 of proliferating cell nuclear antigen (PCNA) is SUMOylated during the physiological cell cycle. To explore the role for SUMOylation of PCNA in DDT, we characterized chicken DT40 and human TK6 B cells deficient in the PIAS1 and PIAS4 small ubiquitin-like modifier (SUMO) E3 ligases. DT40 cells have a unique advantage in the phenotypic analysis of DDT as they continuously diversify their immunoglobulin (Ig) variable genes by TLS and TS [Ig gene conversion (GC)], both relieving replication blocks at abasic sites without accompanying by DNA breakage. Remarkably, PIAS1 (-/-) /PIAS4 (-/-) cells displayed a multifold decrease in SUMOylation of PCNA at Lys164 and over a 90% decrease in the rate of TS. Likewise, PIAS1 (-/-) /PIAS4 (-/-) TK6 cells showed a shift of DDT from TS to TLS at a chemosynthetic UV lesion inserted into the genomic DNA. The PCNA (K164R/K164R) mutation caused a approximately 90% decrease in the rate of Ig GC and no additional impact on PIAS1 (-/-) /PIAS4 (-/-) cells. This epistatic relationship between the PCNA (K164R/K164R) and the PIAS1 (-/-) /PIAS4 (-/-) mutations suggests that PIAS1 and PIAS4 promote TS mainly through SUMOylation of PCNA at Lys164. This idea is further supported by the data that overexpression of a PCNA-SUMO1 chimeric protein restores defects in TS in PIAS1 (-/-) /PIAS4 (-/-) cells. In conclusion, SUMOylation of PCNA at Lys164 promoted by PIAS1 and PIAS4 ensures the error-free release of replication blockage during physiological DNA replication in metazoan cells.

# Orthogonal Cas9-Cas9 chimeras provide a versatile platform for genome editing.

2018

Nature communications

Bolukbasi, Mehmet Fatih

Liu, Pengpeng

Luk, Kevin

Kwok, Samantha F

Gupta, Ankit

Amrani, Nadia

Sontheimer, Erik J

Zhu, Lihua Julie

Wolfe, Scot A

The development of robust, versatile and accurate toolsets is critical to facilitate therapeutic genome editing applications. Here we establish RNA-programmable Cas9-Cas9 chimeras, in single- and dual-nuclease formats, as versatile genome engineering systems. In both of these formats, Cas9-Cas9 fusions display an expanded targeting repertoire and achieve highly specific genome editing. Dual-nuclease Cas9-Cas9 chimeras have distinct advantages over monomeric Cas9s including higher target site activity and the generation of predictable precise deletion products between their target sites. At a therapeutically relevant site within the BCL11A erythroid enhancer, Cas9-Cas9 nucleases produced precise deletions that comprised up to 97% of all sequence alterations. Thus Cas9-Cas9 chimeras represent an important tool that could be particularly valuable for therapeutic genome editing applications where a precise cleavage position and defined sequence end products are desirable.

# New treatments for the mucopolysaccharidoses: from pathophysiology to therapy.

2018

Italian journal of pediatrics

Fecarotta, Simona

Gasperini, Serena

Parenti, Giancarlo

Enzyme replacement therapy is currently considered the standard of care for the treatment of mucopolysaccharidoses (MPS) type I, II, VI, and IV. This approach has shown substantial efficacy mainly on somatic symptoms of the patients, but no benefit was found for other clinical manifestations, such as neurological involvement. New strategies are currently being tested to address these limitations, in particular to obtain sufficient therapeutic levels in the brain. Intrathecal delivery of recombinant enzymes or chimeric enzymes represent promising approaches in this respect. Further innovation will likely be introduced by the recent advancements in the knowledge of lysosomal biology and function. It is now clear that the clinical manifestations of MPS are not only the direct effects of storage, but also derive from a cascade of secondary events that lead to dysfunction of several cellular processes and pathways. Some of these pathways may represent novel therapeutic targets and allow for development of novel or adjunctive therapies for these disorders.

# Covalent capture of OGT's active site using engineered human-E. coli chimera and intrastrand DNA cross-links.

2018

Organic & biomolecular chemistry

Copp, William

O'Flaherty, Derek K

Wilds, Christopher J

O 6-Alkylguanine DNA alkyltransferases (AGTs) are proteins found in most organisms whose role is to remove alkylation damage from the O6- and O4-positions of 2'-deoxyguanosine (dG) and thymidine (dT), respectively. Variations in active site residues between AGTs from different organisms leads to differences in repair proficiency: The human variant (hAGT) has a proclivity for removal of alkyl groups at the O6-position of guanine and the E. coli OGT protein has activity towards the O4-position of thymine. A chimeric protein (hOGT) that our laboratory has engineered with twenty of the active site residues mutated in hAGT to those found in OGT, exhibited activity towards a broader range of substrates relative to native OGT. Among the substrates that the hOGT protein was found to act upon was interstrand cross-linked DNA connected by an alkylene linkage at the O6-position of dG to the complementary strand. In the present study the activity of hOGT towards DNA containing alkylene intrastrand cross-links (IaCL) at the O6- and O4-positions respectively of dG and dT, which lack a phosphodiester linkage between the connected residues, was evaluated. The hOGT protein exhibited proficiency at removal of an alkylene linkage at the O6-atom of dG but the O4-position of dT was refractory to protein activity. The activity of the chimeric hOGT protein towards these IaCLs to prepare well defined DNA-protein cross-linked conjugates will enable mechanistic and high resolution structural studies to address the differences observed in the repair adeptness of O4-alkylated dT by the OGT protein relative to other AGT variants.

# Revisiting the association of HLA alleles and haplotypes with CYP21A2 mutations in a large cohort of patients with congenital adrenal hyperplasia.

2018

Gene

Jayakrishnan, Rahul

Lao, Qizong

Adams, Sharon D

Ward, William W

Merke, Deborah P

The CYP21A2 gene encoding 21hydroxylase is on chromosome 6p21.3 within the human leukocyte antigen (HLA) class III major histocompatibility complex and an association between congenital adrenal hyperplasia (CAH) due to 21hydroxylase deficiency and HLA class I and II alleles has been shown in genetically isolated populations. One-third of CAH causing alleles are 30-kb deletions due to homologous recombination events between active and pseudogenes resulting in chimeric genes. The aim of this study was to re-visit the association between the CYP21A2 variants and HLA polymorphisms in a large ethnically diverse cohort of patients with CAH who underwent comprehensive CYP21A2 genotyping, including specification of chimeric gene subtypes (CAH CH-1 through CH-9 of CYP21A1P/CYP21A2 chimeras; CAH-X CH-1 through CH-3 of TNXA/TNXB chimeras) in alleles with 30-kb deletions. The study population included 201 patients (86 males, 115 females, age 3-75years) with CAH due to 21hydroxylase deficiency (159 classic, 42 nonclassic) and 194 parents. Based on the availability of parental genotype, we determined the haplotypes of CYP21A2 mutations and HLA types in 95 probands (190 alleles). Five prevalent haplotype associations were found: p.V281L and B\*14-C\*08 (P<0.0001); p.I172N and DQB1\*03 (P=0.035); and of the chimeric genes caused by 30-kb deletions: CH-1 and A\*03 (P=0.033); CH-5 and C\*06-DRB1\*07 (P<0.0001); and CAH-X CH-1 and DQB1\*03 (P=0.004). Our findings show that a number of associations between HLA alleles and haplotypes and CYP21A2 mutations, including large 30-kb deletions, exist commonly across ethnicities. These HLA associations may have clinical implications for patients with CAH and may provide insight into the genetics of this highly complex region of the human genome.

# HIV-1 capsids from B27/B57+ elite controllers escape Mx2 but are targeted by TRIM5alpha, leading to the induction of an antiviral state.

2018

PLoS pathogens

Merindol, Natacha

El-Far, Mohamed

Sylla, Mohamed

Masroori, Nasser

Dufour, Caroline

Li, Jia-Xin

Cherry, Pearl

Plourde, Melodie B

Tremblay, Cecile

Berthoux, Lionel

Elite controllers (ECs) are a rare subset of HIV-1 slow progressors characterized by prolonged viremia suppression. HLA alleles B27 and B57 promote the cytotoxic T lymphocyte (CTL)-mediated depletion of infected cells in ECs, leading to the emergence of escape mutations in the viral capsid (CA). Whether those mutations modulate CA detection by innate sensors and effectors is poorly known. Here, we investigated the targeting of CA from B27/B57+ individuals by cytosolic antiviral factors Mx2 and TRIM5alpha. Toward that aim, we constructed chimeric HIV-1 vectors using CA isolated from B27/B57+ or control subjects. HIV-1 vectors containing B27/B57+-specific CA had increased sensitivity to TRIM5alpha but not to Mx2. Following exposure to those vectors, cells showed increased resistance against both TRIM5alpha-sensitive and -insensitive HIV-1 strains. Induction of the antiviral state did not require productive infection by the TRIM5alpha-sensitive virus, as shown using chemically inactivated virions. Depletion experiments revealed that TAK1 and Ubc13 were essential to the TRIM5alpha-dependent antiviral state. Accordingly, induction of the antiviral state was accompanied by the activation of NF-kappaB and AP-1 in THP-1 cells. Secretion of IFN-I was involved in the antiviral state in THP-1 cells, as shown using a receptor blocking antibody. This work identifies innate activation pathways that are likely to play a role in the natural resistance to HIV-1 progression in ECs.

# Arabidopsis thaliana NGATHA1 transcription factor induces ABA biosynthesis by activating NCED3 gene during dehydration stress.

2018

Proceedings of the National Academy of Sciences of the United States of America

Sato, Hikaru

Takasaki, Hironori

Takahashi, Fuminori

Suzuki, Takamasa

Iuchi, Satoshi

Mitsuda, Nobutaka

Ohme-Takagi, Masaru

Ikeda, Miho

Seo, Mitsunori

Yamaguchi-Shinozaki, Kazuko

Shinozaki, Kazuo

The plant hormone abscisic acid (ABA) is accumulated after drought stress and plays critical roles in the responses to drought stress in plants, such as gene regulation, stomatal closure, seed maturation, and dormancy. Although previous reports revealed detailed molecular roles of ABA in stress responses, the factors that contribute to the drought-stress responses-in particular, regulation of ABA accumulation-remain unclear. The enzyme NINE-CIS-EPOXYCAROTENOID DIOXYGENASE 3 (NCED3) is essential for ABA biosynthesis during drought stress, and the NCED3 gene is highly induced by drought stress. In the present study, we isolated NGATHAs (NGAs) as candidate transcriptional regulators of NCED3 through a screen of a plant library harboring the transcription factors fused to a chimeric repressor domain, SRDX. The NGA proteins were directly bound to a cis-element NGA-binding element (NBE) in the 5' untranslated region (5' UTR) of the NCED3 promoter and were suggested to be transcriptional activators of NCED3 Among the single-knockout mutants of four NGA family genes, we found that the NGATHA1 (NGA1) knockout mutant was drought-stress-sensitive with a decreased expression level of NCED3 during dehydration stress. These results suggested that NGA1 essentially functions as a transcriptional activator of NCED3 among the NGA family proteins. Moreover, the NGA1 protein was degraded under nonstressed conditions, and dehydration stress enhanced the accumulation of NGA1 proteins, even in ABA-deficient mutant plants, indicating that there should be ABA-independent posttranslational regulations. These findings emphasize the regulatory mechanisms of ABA biosynthesis during early drought stress.

# HEXIM1-Tat chimera inhibits HIV-1 replication.

2018

PLoS pathogens

Leoz, Marie

Kukanja, Petra

Luo, Zeping

Huang, Fang

Cary, Daniele C

Peterlin, B Matija

Fujinaga, Koh

Transcription of HIV provirus is a key step of the viral cycle, and depends on the recruitment of the cellular positive transcription elongation factor b (P-TEFb) to the HIV promoter. The viral transactivator Tat can displace P-TEFb from the 7SK small nuclear ribonucleoprotein, where it is bound and inactivated by HEXIM1, and bring it to TAR, which allows the stalled RNA polymerase II to transition to successful transcription elongation. In this study, we designed a chimeric inhibitor of HIV transcription by combining functional domains from HEXIM1 and Tat. The chimera (HT1) potently inhibited gene expression from the HIV promoter, by competing with Tat for TAR and P-TEFb binding, while keeping the latter inactive. HT1 inhibited spreading infection as well as viral reactivation in lymphocyte T cell line models of HIV latency, with little effect on cellular transcription and metabolism. This proof-of-concept study validates an innovative approach to interfering with HIV transcription via peptide mimicry and competition for RNA-protein interactions. HT1 represents a new candidate for HIV therapy, or HIV cure via the proposed block and lock strategy.

# [Establishment of a suitable control reporter plasmid of a dual luciferase reporter gene system for hormone research in silkworm cell lines].

2018

Sheng wu gong cheng xue bao = Chinese journal of biotechnology

Liu, Hongling

Lin, Ying

Shen, Guanwang

Gu, Jianjian

Zhang, Haiyan

Wu, Jinxin

Xu, Yinying

Long, Wei

Xia, Qingyou

The dual luciferase reporter gene system provides sensitive readout, while it relies on a constitutively-expressed control gene for readout normalization. However, most standard control reporter genes are not constitutively expressed under all conditions. Here, we report an effective method to construct a control reporter plasmid for the dual luciferase reporter gene system that would be suitable for hormone research in silkworm cell lines. First, we modified BmVgP78M, a stably-expressed constitutive promoter in silkworm cells by mutating its hormone-related element. Then, we constructed the pRL-VgP78M control reporter plasmid by replacing the SV40 promoter and chimeric intron sequences in pRL-SV40 with the BmVgP78M sequence. Finally, we confirmed that the pRL-VgP78M control reporter plasmid could be stably expressed in silkworm cell lines via cell transfection experiments, and it was unresponsive to the induction of ecdysone, juvenile hormone, or their transcription factors. We thus obtained a control reporter plasmid pRL-VgP78M that could be expressed stably and moderately in silkworm cells. It can be readily used as the control reporter plasmid of the dual luciferase reporter gene system for hormone research in silkworm cell lines. It will also provide a reference for construction of control reporter plasmids of dual luciferase reporter gene systems that are adaptable to cell lines isolated from other species.

# Selectivity and Promiscuity in TET-Mediated Oxidation of 5-Methylcytosine in DNA and RNA.

2018

Biochemistry

DeNizio, Jamie E

Liu, Monica Yun

Leddin, Emmett M

Cisneros, G Andres

Kohli, Rahul M

Enzymes of the ten-eleven translocation (TET) family add diversity to the repertoire of nucleobase modifications by catalyzing the oxidation of 5-methylcytosine (5mC). TET enzymes were initially found to oxidize 5-methyl-2'-deoxycytidine in genomic DNA, yielding products that contribute to epigenetic regulation in mammalian cells, but have since been found to also oxidize 5-methylcytidine in RNA. Considering the different configurations of single-stranded (ss) and double-stranded (ds) DNA and RNA that coexist in a cell, defining the scope of TET's preferred activity and the mechanisms of substrate selectivity is critical to better understand the enzymes' biological functions. To this end, we have systematically examined the activity of human TET2 on DNA, RNA, and hybrid substrates in vitro. We found that, while ssDNA and ssRNA are well tolerated, TET2 is most proficient at dsDNA oxidation and discriminates strongly against dsRNA. Chimeric and hybrid substrates containing mixed DNA and RNA character helped reveal two main features by which the enzyme discriminates between substrates. First, the identity of the target nucleotide alone is the strongest reactivity determinant, with a preference for 5-methyldeoxycytidine, while both DNA or RNA are relatively tolerated on the rest of the target strand. Second, while a complementary strand is not required for activity, DNA is the preferred partner, and complementary RNA diminishes reactivity. Our biochemical analysis, complemented by molecular dynamics simulations, provides support for an active site optimally configured for dsDNA reactivity but permissive for various nucleic acid configurations, suggesting a broad range of plausible roles for TET-mediated 5mC oxidation in cells.

# Cancer diagnosis and immunotherapy in the age of CRISPR.

2018

Genes, chromosomes & cancer

Cook, Peter J

Ventura, Andrea

The explosion in genome editing technologies that has occurred in the past decade has revolutionized cancer research and promises to improve cancer diagnosis and therapy. Ongoing efforts include engineering of chimeric antigen receptor-T cells using clustered regularly interspaced short palindromic repeats (CRISPR) to generate a safer, more effective therapy with improved performance in immunologically "cold" tumors, as well as clever adaptations of CRISPR enzymes to allow fast, simple, and sensitive detection of specific nucleotide sequences. While still in their infancy, CRISPR-based cancer therapeutics and diagnostics are developing at an impressive speed and it is likely they will soon impact clinical practice. Here, we summarize their history and the most recent developments.

# Different Degradation Mechanisms of Inhibitor of Apoptosis Proteins (IAPs) by the Specific and Nongenetic IAP-Dependent Protein Eraser (SNIPER).

2018

Chemical & pharmaceutical bulletin

Ohoka, Nobumichi

Ujikawa, Osamu

Shimokawa, Kenichiro

Sameshima, Tomoya

Shibata, Norihito

Hattori, Takayuki

Nara, Hiroshi

Cho, Nobuo

Naito, Mikihiko

Targeted protein degradation by small molecules is an emerging modality with significant potential for drug discovery. We previously developed chimeric molecules, termed specific and non-genetic inhibitor of apoptosis protein (IAP)-dependent protein erasers (SNIPERs), which induce the ubiquitylation and proteasomal degradation of target proteins. This degradation is mediated by the IAPs; the target proteins include bromodomain-containing protein 4 (BRD4), an epigenetic regulator protein. The SNIPER that degrades this particular protein, SNIPER(BRD)-1, consists of an IAP antagonist LCL-161 derivative and a bromodomain and extra-terminal (BET) inhibitor, (+)-JQ-1. SNIPER(BRD)-1 also degrades a cellular inhibitor of apoptosis protein 1 (cIAP1) and an X-linked inhibitor of apoptosis protein (XIAP), the mechanisms of which are not well understood. Here, we show that the degradation of cIAP1 and XIAP by SNIPER(BRD)-1 is induced via different mechanisms. Using a chemical biology-based approach, we developed two inactive SNIPERs, SNIPER(BRD)-3 and SNIPER(BRD)-4, incapable of degrading BRD4. SNIPER(BRD)-3 contained an N-methylated LCL-161 derivative as the IAP ligand, which prevented it from binding IAPs, and resulted in the abrogated degradation of cIAP1, XIAP, and BRD4. SNIPER(BRD)-4, however, incorporated the enantiomer (-)-JQ-1 which was incapable of binding BRD4; this SNIPER degraded cIAP1 but lost the ability to degrade XIAP and BRD4. Furthermore, a mixture of the ligands, (+)-JQ-1 and LCL-161, induced the degradation of cIAP1, but not XIAP and BRD4. These results indicate that cIAP1 degradation is triggered by the binding of the IAP antagonist module to induce autoubiquitylation of cIAP1, whereas a ternary complex formation is required for the SNIPER-induced degradation of XIAP and BRD4.

# Evolutionary Morphing of Tryptophan Synthase: Functional Mechanisms for the Enzymatic Channeling of Indole.

2018

Journal of molecular biology

Fleming, Jennifer R

Schupfner, Michael

Busch, Florian

Basle, Arnaud

Ehrmann, Alexander

Sterner, Reinhard

Mayans, Olga

Tryptophan synthase (TrpS) is a heterotetrameric alphabetabetaalpha enzyme that exhibits complex substrate channeling and allosteric mechanisms and is a model system in enzymology. In this work, we characterize proposed early and late evolutionary states of TrpS and show that they have distinct quaternary structures caused by insertions-deletions of sequence segments (indels) in the beta-subunit. Remarkably, indole hydrophobic channels that connect alpha and beta active sites have re-emerged in both TrpS types, yet they follow different paths through the beta-subunit fold. Also, both TrpS geometries activate the alpha-subunit through the rearrangement of loops flanking the active site. Our results link evolutionary sequence changes in the enzyme subunits with channeling and allostery in the TrpS enzymes. The findings demonstrate that indels allow protein quaternary architectures to escape "minima" in the evolutionary landscape, thereby overcoming the conservational constraints imposed by existing functional interfaces and being free to morph into new mechanistic enzymes.

# Transient Retrovirus-Based CRISPR/Cas9 All-in-One Particles for Efficient, Targeted Gene Knockout.

2018

Molecular therapy. Nucleic acids

Knopp, Yvonne

Geis, Franziska K

Heckl, Dirk

Horn, Stefan

Neumann, Thomas

Kuehle, Johannes

Meyer, Janine

Fehse, Boris

Baum, Christopher

Morgan, Michael

Meyer, Johann

Schambach, Axel

Galla, Melanie

The recently discovered CRISPR/Cas9 system is widely used in basic research and is a useful tool for disease modeling and gene editing therapies. However, long-term expression of DNA-modifying enzymes can be associated with cytotoxicity and is particularly unwanted in clinical gene editing strategies. Because current transient expression methods may still suffer from cytotoxicity and/or low efficiency, we developed non-integrating retrovirus-based CRISPR/Cas9 all-in-one particles for targeted gene knockout. By redirecting the gammaretroviral packaging machinery, we transiently delivered Streptococcus pyogenes Cas9 (SpCas9) mRNA and single-guide RNA transcripts into various (including primary) cell types. Spatiotemporal co-delivery of CRISPR/Cas9 components resulted in efficient disruption of a surrogate reporter gene, as well as functional knockout of endogenous human genes CXCR4 and TP53. Although acting in a hit-and-run fashion, knockout efficiencies of our transient particles corresponded to 52%-80% of those obtained from constitutively active integrating vectors. Stable SpCas9 overexpression at high doses in murine NIH3T3 cells caused a substantial G0/G1 arrest accompanied by reduced cell growth and metabolic activity, which was prevented by transient SpCas9 transfer. In summary, the non-integrating retrovirus-based vector particles introduced here allow efficient and dose-controlled delivery of CRISPR/Cas9 components into target cells.

# Characterization of the catalytic flexible loop in the dihydroorotase domain of the human multi-enzymatic protein CAD.

2018

The Journal of biological chemistry

Del Cano-Ochoa, Francisco

Grande-Garcia, Araceli

Reverte-Lopez, Maria

D'Abramo, Marco

Ramon-Maiques, Santiago

The dihydroorotase (DHOase) domain of the multifunctional protein carbamoyl-phosphate synthetase 2, aspartate transcarbamoylase, and dihydroorotase (CAD) catalyzes the third step in the de novo biosynthesis of pyrimidine nucleotides in animals. The crystal structure of the DHOase domain of human CAD (huDHOase) revealed that, despite evolutionary divergence, its active site components are highly conserved with those in bacterial DHOases, encoded as monofunctional enzymes. An important element for catalysis, conserved from Escherichia coli to humans, is a flexible loop that closes as a lid over the active site. Here, we combined mutagenic, structural, biochemical, and molecular dynamics analyses to characterize the function of the flexible loop in the activity of CAD's DHOase domain. A huDHOase chimera bearing the E. coli DHOase flexible loop was inactive, suggesting the presence of distinctive elements in the flexible loop of huDHOase that cannot be replaced by the bacterial sequence. We pinpointed Phe-1563, a residue absolutely conserved at the tip of the flexible loop in CAD's DHOase domain, as a critical element for the conformational equilibrium between the two catalytic states of the protein. Substitutions of Phe-1563 with Ala, Leu, or Thr prevented the closure of the flexible loop and inactivated the protein, whereas substitution with Tyr enhanced the interactions of the loop in the closed position and reduced fluctuations and the reaction rate. Our results confirm the importance of the flexible loop in CAD's DHOase domain and explain the key role of Phe-1563 in configuring the active site and in promoting substrate strain and catalysis.

# Enzyme Fusion Removes Competition for Geranylgeranyl Diphosphate in Carotenogenesis.

2018

Plant physiology

Camagna, Maurizio

Grundmann, Alexander

Bar, Cornelia

Koschmieder, Julian

Beyer, Peter

Welsch, Ralf

Geranylgeranyl diphosphate (GGPP), a prenyl diphosphate synthesized by GGPP synthase (GGPS), represents a metabolic hub for the synthesis of key isoprenoids, such as chlorophylls, tocopherols, phylloquinone, gibberellins, and carotenoids. Protein-protein interactions and the amphipathic nature of GGPP suggest metabolite channeling and/or competition for GGPP among enzymes that function in independent branches of the isoprenoid pathway. To investigate substrate conversion efficiency between the plastid-localized GGPS isoform GGPS11 and phytoene synthase (PSY), the first enzyme of the carotenoid pathway, we used recombinant enzymes and determined their in vitro properties. Efficient phytoene biosynthesis via PSY strictly depended on simultaneous GGPP supply via GGPS11. In contrast, PSY could not access freely diffusible GGPP or time-displaced GGPP supply via GGPS11, presumably due to liposomal sequestration. To optimize phytoene biosynthesis, we applied a synthetic biology approach and constructed a chimeric GGPS11-PSY metabolon (PYGG). PYGG converted GGPP to phytoene almost quantitatively in vitro and did not show the GGPP leakage typical of the individual enzymes. PYGG expression in Arabidopsis resulted in orange-colored cotyledons, which are not observed if PSY or GGPS11 are overexpressed individually. This suggests insufficient GGPP substrate availability for chlorophyll biosynthesis achieved through GGPP flux redirection to carotenogenesis. Similarly, carotenoid levels in PYGG-expressing callus exceeded that in PSY- or GGPS11-overexpression lines. The PYGG chimeric protein may assist in provitamin A biofortification of edible plant parts. Moreover, other GGPS fusions may be used to redirect metabolic flux into the synthesis of other isoprenoids of nutritional and industrial interest.

# Chimeric padlock and iLock probes for increased efficiency of targeted RNA detection.

2018

RNA (New York, N.Y.)

Krzywkowski, Tomasz

Kuhnemund, Malte

Nilsson, Mats

Many approaches exist to detect RNA using complementary oligonucleotides. DNA ligation-based techniques can improve discrimination of subtle sequence variations, but they have been difficult to implement for direct RNA analysis due to the infidelity and inefficiency of most DNA ligases on RNA. In this report, we have systematically studied if ribonucleotide substitutions in padlock probes can provide higher catalytic efficiencies for Chlorella virus DNA ligase (PBCV-1 DNA ligase) and T4 RNA ligase 2 (T4Rnl2) on RNA. We provide broad characterization of end-joining fidelity for both enzymes in RNA-templated 3'-OH RNA/5'-pDNA chimeric probe ligation. Both ligases showed increased ligation efficiency toward chimeric substrates on RNA. However, end-joining fidelity of PBCV-1 DNA ligase remained poor, while T4Rnl2 showed a somewhat better end-joining fidelity compared to PBCV-1 DNA ligase. The recently presented invader padlock (iLock) probes overcome the poor end-joining fidelity of PBCV-1 DNA ligase by the requirement of target-dependent 5' flap removal prior to ligation. Here we show that two particular ribonucleotide substitutions greatly improve the activation and ligation rate of chimeric iLock probes on RNA. We characterized the end-joining efficiency and fidelity of PBCV-1 DNA ligase and T4Rnl2 with chimeric iLock probes on RNA and found that both enzymes exhibit high ligation fidelities for single nucleotide polymorphisms on RNA. Finally, we applied the chimeric probe concept to directly differentiate between human and mouse ACTB mRNA in situ, demonstrating chimeric padlock and iLock probes as superior to their DNA equivalents.

# Crystal structure of a human ubiquitin E1-ubiquitin complex reveals conserved functional elements essential for activity.

2018

The Journal of biological chemistry

Lv, Zongyang

Williams, Katelyn M

Yuan, Lingmin

Atkison, James H

Olsen, Shaun K

Ubiquitin (Ub) signaling plays a key regulatory role in nearly every aspect of eukaryotic biology and is initiated by E1 enzymes that activate and transfer Ub to E2 Ub-conjugating enzymes. Despite Ub E1's fundamental importance to the cell and its attractiveness as a target for therapeutic intervention in cancer and other diseases, its only available structural information is derived from yeast orthologs of human ubiquitin-like modifier-activating enzyme 1 (hUBA1). To illuminate structural differences between yeast and hUBA1 structures that might be exploited for the development of small-molecule therapeutics, we determined the first crystal structure of a hUBA1-Ub complex. Using structural analysis, molecular modeling, and biochemical analysis, we demonstrate that hUBA1 shares a conserved overall structure and mechanism with previously characterized yeast orthologs, but displays subtle structural differences, particularly within the active site. Computational analysis revealed four potential ligand-binding hot spots on the surface of hUBA1 that might serve as targets to inhibit hUBA1 at the level of Ub activation or E2 recruitment or that might potentially be used in approaches such as protein-targeting chimeric molecules. Taken together, our work enhances our understanding of the hUBA1 mechanism, provides an improved framework for the development of small-molecule inhibitors of UBA1, and serves as a stepping stone for structural studies that involve the enzymes of the human Ub system at the level of both E1 and E2.

# Light control of G protein signaling pathways by a novel photopigment.

2018

PloS one

Osorno, Tomas

Arenas, Oscar

Ramirez-Suarez, Nelson J

Echeverry, Fabio A

Gomez, Maria Del Pilar

Nasi, Enrico

Channelopsins and photo-regulated ion channels make it possible to use light to control electrical activity of cells. This powerful approach has lead to a veritable explosion of applications, though it is limited to changing membrane voltage of the target cells. An enormous potential could be tapped if similar opto-genetic techniques could be extended to the control of chemical signaling pathways. Photopigments from invertebrate photoreceptors are an obvious choice-as they do not bleach upon illumination -however, their functional expression has been problematic. We exploited an unusual opsin, pScop2, recently identified in ciliary photoreceptors of scallop. Phylogenetically, it is closer to vertebrate opsins, and offers the advantage of being a bi-stable photopigment. We inserted its coding sequence and a fluorescent protein reporter into plasmid vectors and demonstrated heterologous expression in various mammalian cell lines. HEK 293 cells were selected as a heterologous system for functional analysis, because wild type cells displayed the largest currents in response to the G-protein activator, GTP-gamma-S. A line of HEK cells stably transfected with pScop2 was generated; after reconstitution of the photopigment with retinal, light responses were obtained in some cells, albeit of modest amplitude. In native photoreceptors pScop2 couples to Go; HEK cells express poorly this G-protein, but have a prominent Gq/PLC pathway linked to internal Ca mobilization. To enhance pScop2 competence to tap into this pathway, we swapped its third intracellular loop-important to confer specificity of interaction between 7TMDRs and G-proteins-with that of a Gq-linked opsin which we cloned from microvillar photoreceptors present in the same retina. The chimeric construct was evaluated by a Ca fluorescence assay, and was shown to mediate a robust mobilization of internal calcium in response to illumination. The results project pScop2 as a potentially powerful optogenetic tool to control signaling pathways.

# Bortezomib improves adoptive carbonic anhydrase IXspecific chimeric antigen receptormodified NK92 cell therapy in mouse models of human renal cell carcinoma.

2018

Oncology reports

Zhang, Qing

Xu, Jinjing

Ding, Jiage

Liu, Hongyan

Li, Huizhong

Li, Hailong

Lu, Mengmeng

Miao, Yangna

Wang, Zhenzhen

Fu, Qiang

Zheng, Junnian

Adoptive chimeric antigen receptor (CAR) T or NK cells offer new options for cancer treatment. Clinical results indicate that CARmodified T cell (CART) therapy has curative therapeutic efficacy for hematological malignancies. However, the efficacy of the therapy in most solid tumors, including advanced renal cell carcinoma (RCC), remains highly limited. New regimens, including combination of CART cells with chemical drugs, must be studied to enhance the therapeutic efficacy of CART or NK cells for solid tumors. In the present study, a carbonic anhydrase IX (CAIX)specific thirdgeneration CAR was transduced into NK92 cells by lentiviral vectors. The immune effects, including cytokine release and cytotoxicity, of the CARNK92 cells against CAIXpositive RCC cells were evaluated in vitro. Combination therapeutic effects of bortezomib and CARNK92 cells were analyzed in a mouse model with human RCC xenografts. The results revealed that CAIXspecific CARNK92 cells specifically recognized in vitro cultured CAIXpositive RCC cells and released cytokines, including IFNgamma, perforin and granzyme B, and exhibited specific cytotoxicity. The cytotoxicity of the CARNK92 cells was enhanced after treating RCC cells with bortezomib in vitro. The suppressive efficacy of bortezomib combined with CARNK92 cells against established CAIXpositive tumor xenografts was more significant than that of the monotherapy with either CARNK92 cells or bortezomib. Therefore, bortezomib can enhance the effects of the CARNK92 cells against RCC in vitro and in vivo. This study provided an experimental basis for the novel clinical regimen of CAIXspecific CARmodified NK or T cells for the treatment of RCC.

# Designing and Expression of Recombinant Chimeric Protein Containing CtxB and OmpW from Vibrio Cholerae and Evaluation of Its Immunogenicity.

2018

Iranian journal of immunology : IJI

Vakili, Atina

Mousavi Gargari, Seyed Latif

Nazarian, Shahram

Amani, Jafar

BACKGROUND: Cholera disease caused by Vibrio cholerae remains a major cause of morbidity and mortality throughout the world. Various strategies with different proteins as immunogens have been tried for vaccine development, none of which have been sufficiently effective to preclude cholera. Chimeric proteins, with their ability to present multiple antigens at the same time, can play important roles in immunization. OBJECTIVE: To evaluate the immunogenicity of a chimeric construct, comprised of OmpW and CtxB as immunogenic proteins of Vibrio cholera, in BALB/c mice. METHODS: The construct was designed after bioinformatics assessments and then expressed in E.coli. Chimeric protein, OmpW, and CtxB were purified with Ni-NTA chromatography and confirmed by Western blotting. Mice were immunized with purified recombinant proteins. The antibody titers and specificity of the immune sera were then analyzed by ELISA and challenged on the pups of immunized mice with 1, 5 and 10 LD50. Mice ileal loop assay was also performed. RESULTS: Significant differences were observed in antibody titers in immunized mice compared to the control groups. Infant mouse challenge was performed so as to compare the protective efficacies of the selected immunogen regimens. Of the Pups from dams immunized with chimeric protein which received 1 LD50, 75% survived. Pups belonging to PBS-immunized dams, experienced 100% mortality. The serum raised toward immunogenic construct, inhibited cholera toxin activity in ileal loop test up to 68%. CONCLUSION: Chimeric construct is able to induce the immune system and provide up to 75% inhibition of toxin activity against 1 LD50 of Vibrio cholerae.

# Designing DNAzyme-Powered Nanomachines Simultaneously Responsive to Multiple MicroRNAs.

2018

Chemistry (Weinheim an der Bergstrasse, Germany)

Zhong, Xiaoxi

Yang, Sishu

Yang, Peng

Du, Huan

Hou, Xiandeng

Chen, Junbo

Zhou, Rongxing

Herein, a DNAzyme-powered nanomachine responsive to multiple hepatocellular carcinoma (HCC)-related miRNAs derived from clinical samples was designed. Initially, three types of nanomachines were constructed with dye molecule [(fluorescein (FAM), tetramethylrhodamin (TMR), and Cyanine 5 (Cy5)]-labeled DNA-RNA chimeric substrates and a specific recognized probe for the corresponding miRNAs target. Once the target miRNAs were captured by two recognizing probes, the DNA nanomachine was initiated, leading to the hybridization between the DNAzyme and the substrates. With the help of a cofactor, the automatic operation of the nanomachine was driven by cyclic cleavage of the DNAzyme. Meanwhile, we also explored the recognition behavior between the recognizing probe and the target miRNA. Subsequently, these DNAzyme-powered nanomachines were developed for the homogeneous and simultaneous detection of three target miRNAs at the femtomloar level. Furthermore, the potential in clinical diagnosis was proven by the successful determination of target miRNA in real clinical samples. Thus, this nanomachine-based strategy possesses significant potential to be an innovation in miRNA analysis methodology.

# Gi/o-coupled muscarinic receptors co-localize with GIRK channel for efficient channel activation.

2018

PloS one

Tateyama, Michihiro

Kubo, Yoshihiro

G protein-gated inwardly rectifying K+ (GIRK) channel regulates cellular excitability upon activation of Gi/o-coupled receptors. In Gi/o-coupled muscarinic M2R, the intracellular third loop (i3) is known as a key domain for Gi/o coupling, because replacement of i3 of Gq-coupled muscarinic M1R with that of M2R enables the chimeric receptor (MC9) to activate the GIRK channel. In the present study, we showed that MC9, but not M1R, co-localizes with the GIRK channel and Galphai1 by Forster resonance energy transfer (FRET) analysis. When M1R was forced to stay adjacent to the channel through ligation with short linkers, M1R activated the GIRK channel. FRET analysis further suggested that the efficacy of channel activation is correlated with the linker length between M1R and the GIRK channel. The results show that co-localization is an important factor for activating the GIRK channel. In contrast, for MC9 and M2R, the GIRK channel was activated even when they were connected by long linkers, suggesting the formation of a molecular complex even in the absence of a linker. We also observed that replacement of 13 amino acid residues at the N-terminal end of i3 of MC9 with those of M1R impaired the co-localization with the GIRK channel as well as channel activation. These results show that localization of the receptor near the GIRK channel is a key factor in efficiently activating the channel and that the N-terminal end of i3 of M2R plays an important role in co-localization.

# Alpha kinase 1 controls intestinal inflammation by suppressing the IL-12/Th1 axis.

2018

Nature communications

Ryzhakov, Grigory

West, Nathaniel R

Franchini, Fanny

Clare, Simon

Ilott, Nicholas E

Sansom, Stephen N

Bullers, Samuel J

Pearson, Claire

Costain, Alice

Vaughan-Jackson, Alun

Goettel, Jeremy A

Ermann, Joerg

Horwitz, Bruce H

Buti, Ludovico

Lu, Xin

Mukhopadhyay, Subhankar

Snapper, Scott B

Powrie, Fiona

Inflammatory bowel disease (IBD) are heterogenous disorders of the gastrointestinal tract caused by a spectrum of genetic and environmental factors. In mice, overlapping regions of chromosome 3 have been associated with susceptibility to IBD-like pathology, including a locus called Hiccs. However, the specific gene that controls disease susceptibility remains unknown. Here we identify a Hiccs locus gene, Alpk1 (encoding alpha kinase 1), as a potent regulator of intestinal inflammation. In response to infection with the commensal pathobiont Helicobacter hepaticus (Hh), Alpk1-deficient mice display exacerbated interleukin (IL)-12/IL-23 dependent colitis characterized by an enhanced Th1/interferon(IFN)-gamma response. Alpk1 controls intestinal immunity via the hematopoietic system and is highly expressed by mononuclear phagocytes. In response to Hh, Alpk1(-/-) macrophages produce abnormally high amounts of IL-12, but not IL-23. This study demonstrates that Alpk1 promotes intestinal homoeostasis by regulating the balance of type 1/type 17 immunity following microbial challenge.

# EGF ligand fused to truncated Pseudomonas aeruginosa exotoxin A specifically targets and inhibits EGFRpositive cancer cells.

2018

Oncology reports

Hashimi, Saeed M

Grant, Brock

Alqurashi, Naif

Alowaidi, Faisal

Wei, Ming Q

Cancer cells have been known to overexpress the epidermal growth factor receptor (EGFR) and hence relevant multipletargeted therapies have been developed, with a recent clinical application of the antibodymediated inhibition of the EGFR. However, this strategy is not useful in cancer cells with mutations in KRAS; a GTPase downstream of EGFR which constitutively activates the pathway without EGF stimulation. Furthermore, mutations in EGFR also reduce the binding of monoclonal antibodies and thereby render them ineffective. In the present study, we designed a chimeric EGF protein fused to the truncated Nterminal domain fragment of Pseudomonas aeruginosa exotoxin A (EGFETA), which has ADPribosylation activity and induces apoptosis. The EGFETA protein was expressed in E. coli as a Histagged fusion. Our results showed that EGFETA significantly inhibited the proliferation of EGFRpositive A431 epidermoid carcinoma (IC50 27 ng/ml) and HN5 head and neck squamous cell carcinoma (IC50 36 ng/ml) cells. However, its effect on cancer cells with little or no EGFR expression was limited (A549IC50 1,000 ng/ml; MCF7IC50 >10,000 ng/ml). Compared to cetuximab, EGFETA was highly potent in its killing capacity of HN5 cancer cells at 1,000 ng/ml, while cetuximab had little effect at 1,000 ng/ml. Furthermore, EGFETA was just as potent in HCT116 (KRAS G13D) and SW480 (KRAS G12V) colon cancer cell lines harbouring KRAS hyperactivating mutations when compared to KRAS wildtype HT29 colon cancer cells. Finally, coincubation of EGFETA with an antiEGF antibody abrogated its effect on the EGFRpositive A431 cells. Our results show that the chimeric EGFETA toxin is extremely effective against EGFRpositive cancers and raises the potential to further develop this chimera for use in targeting EGFRpositive tumours resistant to monoclonal antibodies.

# Highly multiplexed genome engineering using CRISPR/Cas9 gRNA arrays.

2018

PloS one

Kurata, Morito

Wolf, Natalie K

Lahr, Walker S

Weg, Madison T

Kluesner, Mitchell G

Lee, Samantha

Hui, Kai

Shiraiwa, Masano

Webber, Beau R

Moriarity, Branden S

The CRISPR/Cas9 system is an RNA guided nuclease system that evolved as a mechanism of adaptive immunity in bacteria. This system has been adopted for numerous genome engineering applications in research and recently, therapeutics. The CRISPR/Cas9 system has been largely implemented by delivery of Cas9 as protein, RNA, or plasmid along with a chimeric crRNA-tracrRNA guide RNA (gRNA) under the expression of a pol III promoter, such as U6. Using this approach, multiplex genome engineering has been achieved by delivering several U6-gRNA plasmids targeting multiple loci. However, this approach is limited due to the efficiently of delivering multiple plasmids to a single cell at one time. To augment the capability and accessibility of multiplexed genome engineering, we developed an efficient golden gate based method to assemble gRNAs linked by optimal Csy4 ribonuclease sequences to deliver up to 10 gRNAs as a single gRNA array transcript. Here we report the optimal expression of our guide RNA array under a strong pol II promoter. This system can be implemented alongside the myriad of CRISPR applications, allowing users to model complex biological processes requiring numerous gRNAs.

# Development of a highly accurate and sensitive diagnostic tool for pyrethroid-resistant chimeric P450 CYP337B3 of Helicoverpa armigera using loop-mediated isothermal amplification.

2018

Archives of insect biochemistry and physiology

Choi, Bo Hey

Hur, Joon Haeng

Heckel, David G

Kim, Juil

Koh, Young Ho

Recent studies have shown that pyrethroid resistance in the cotton bollworm (CBW) Helicoverpa armigera is conferred by the generation of a chimeric CYP337B3 gene, which resulted from unequal crossing-over between the CYP337B1 and CYP337B2 genes. In this study, we developed a diagnostic protocol based on the loop-mediated isothermal amplification (LAMP) assay for the detection of chimeric CYP337B3. The CYP337B3 LAMP assay utilized six primers and generated strong fluorescence signals visible to the naked eye under normal or ultraviolet light. The primers were designed based on CYP337B3v1 (JQ995292), the major allele detected in Australia. The detection limit of this LAMP assay was 10 fg genomic DNA in a 25-microl reaction mixture. Compared with CYP337B2v1, the Korean CYP337B3v2 allele had two nucleotide mismatches within the amplifying regions of this LAMP assay; therefore, we confirmed that polymerase chain reaction-synthesized CYP337B3v2 was well amplified using this LAMP assay. In addition, we determined that the presence of CYP337B3 from H. armigera collected by pheromone traps from Korean fields could be confirmed using this LAMP assay. This assay could detect CYP337B3 even in heterozygotes, which is relevant because CYP337B3 is dominant, and heterozygotes are pyrethroid resistant. Therefore, the newly developed CYP337B3 LAMP assay could detect the presence of pyrethroid resistance in H. armigera that were captured by pheromone traps during the early season and provide information on whether pyrethroids could be used to control H. armigera.

# Translocation-generated ITK-FER and ITK-SYK fusions induce STAT3 phosphorylation and CD69 expression.

2018

Biochemical and biophysical research communications

Fathi, Narmeen N

Mohammad, Dara K

Gorgens, Andre

Andaloussi, Samir El

Zain, Rula

Nore, Beston F

Smith, C I Edvard

Many cancer types carry mutations in protein tyrosine kinase (PTK) and such alterations frequently drive tumor progression. One category is gene translocation of PTKs yielding chimeric proteins with transforming capacity. In this study, we characterized the role of ITK-FER [Interleukin-2-inducible T-cell Kinase (ITK) gene fused with Feline Encephalitis Virus-Related kinase (FER) gene] and ITK-SYK [Interleukin-2-inducible T-cell Kinase (ITK) gene fused with the Spleen Tyrosine Kinase (SYK)] in Peripheral T Cell Lymphoma (PTCL) signaling. We observed an induction of tyrosine phosphorylation events in the presence of both ITK-FER and ITK-SYK. The downstream targets of ITK-FER and ITK-SYK were explored and STAT3 was found to be highly phosphorylated by these fusion kinases. In addition, the CD69 T-cell activation marker was significantly elevated. Apart from tyrosine kinase inhibitors acting directly on the fusions, we believe that drugs acting on downstream targets could serve as alternative cancer therapies for fusion PTKs.

# Targeting CD46 Enhances Anti-Tumoral Activity of Adenovirus Type 5 for Bladder Cancer.

2018

International journal of molecular sciences

Do, Manh-Hung

To, Phuong Kim

Cho, Young-Suk

Kwon, Se-Young

Hwang, Eu Chang

Choi, Chan

Cho, Sang-Hee

Lee, Sang-Jin

Hemmi, Silvio

Jung, Chaeyong

CD46 is generally overexpressed in many human cancers, representing a prime target for CD46-binding adenoviruses (Ads). This could help to overcome low anti-tumoral activity by coxsackie-adenoviral receptor (CAR)-targeting cancer gene therapy viruses. However, because of scarce side-by-side information about CAR and CD46 expression levels in cancer cells, mixed observations of cancer therapeutic efficacy have been observed. This study evaluated Ad-mediated therapeutic efficacy using either CAR-targeting Ad5 or CD46-targeting Ad5/35 fiber chimera in bladder cancer cell lines. Compared with normal urothelia, bladder cancer tissue generally overexpressed both CAR and CD46. While CAR expression was not correlated with disease progression, CD46 expression was inversely correlated with tumor grade, stage, and risk grade. In bladder cancer cell lines, expression levels of CD46 and CAR were highly correlated with Ad5/35- and Ad5-mediated gene transduction and cytotoxicity, respectively. In a human EJ bladder cancer xenograft mouse model, with either overexpressed or suppressed CD46 expression levels, Ad5/35-tk followed by ganciclovir (GCV) treatment significantly affected tumor growth, whereas Ad5-tk/GCV had only minimal effects. Overall, our findings suggest that bladder cancer cells overexpress both CAR and CD46, and that adenoviral cancer gene therapy targeting CD46 represents a more suitable therapy option than a CAR-targeting therapy, especially in patients with low risk bladder cancers.

# Impaired heterologous protein-protein interaction is an essential cause for non-viability of WNV/DENV recombinants.

2018

Virology

Lei, Yingfen

Takeda, Kazuyo

Yu, Li

Flavivirus RNA replication starts at 3'-end, where it folds into a highly conserved stem-loop structure. We attempted to identify the viral non-structural proteins (NSPs) that might specifically interact with the 3'-stemloop (3'SL) through a genetic approach. WNV/DENV2 chimeric recombinants that contain Dengue2 (DENV2) gene(s) in West Nile virus (WNV) backbone were tested for replication competence. Three of seven recombinant viruses, containing the DENV2 NS1, NS2A, or NS4B gene and terminated with a mutated 3'SL (MutC 3'SL), were viable. Of these three, only those bearing the DENV2 NS1 and NS2A substitutions remained infectious when the MutC 3'SL was replaced by the wildtype WNV 3'SL. However, none of the seven chimeric recombinants bearing the DENV2 3'SL were viable. We then investigated the causes for failed replication of WNV/DENV2 chimeric recombinants. Proteolytic cleavage of NS polyproteins was defective by heterologous protease NS2B/3, but was efficient by homologous DENV2 NS2B/3 protease. Whereas, the heterologous polyproteins that contained DENV2 homologous protease were found to produce abnormal vesicles. WNV/DENV2 recombinants expressing the DENV2 homologous protease did not produce infectious virus either. We examined NS protein-protein interaction (PPI) and found that heterologous PPI (hPPI) between WNV and DENV2 NSPs were impaired to various degrees. Insufficient PPIs occurred mainly between heterologous NS2B and NS3; NS2B and NS4A; NS3 and NS5, correlating to those non-viability of substitution mutants. Our results indicate that impaired PPI may decrease protease activity and affect vesicle formation, and is the essential cause for non-viability of the WNV/DENV2 recombinants.

# Novel Human NK Cell Line Carrying CAR Targeting EGFRvIII Induces Antitumor Effects in Glioblastoma Cells.

2018

Anticancer research

Murakami, Toshiharu

Nakazawa, Tsutomu

Natsume, Atsushi

Nishimura, Fumihiko

Nakamura, Mitsutoshi

Matsuda, Ryosuke

Omoto, Koji

Tanaka, Yoshitaka

Shida, Youichi

Park, Young-Soo

Motoyama, Yasushi

Nakagawa, Ichiro

Yamada, Shuichi

Tamura, Kentaro

Takeshima, Yasuhiro

Takamura, Yoshiaki

Wakabayashi, Toshihiko

Nakase, Hiroyuki

BACKGROUND/AIM: Natural killer (NK) cells are considered potential antitumor effector cells. The aim of this study was to establish a novel type of a chimeric antigen receptor (CAR) NK cell line (CAR-KHYG-1) specific for epidermal growth factor receptor variant III (EGFRvIII)-expressing tumors and investigate the anti-tumor activity of EGFRvIII-specific-CAR-KHYG-1 (EvCAR-KHYG-1). MATERIALS AND METHODS: EvCAR-KHYG-1 was established by self-inactivated lentiviral-based transduction of the EvCAR gene and magnetic bead-based purification of EvCAR-expressing NK cells. The anti-tumor effects of EvCAR-KHYG-1 were evaluated using growth inhibition and apoptosis detection assays in glioblastoma (GBM) cell lines (EGFRvIII-expressing and non-expressing U87MG). RESULTS: The findings demonstrated that EvCAR-KHYG-1 inhibited GBM cell-growth via apoptosis in an EGFRvIII-expressing specific manner. CONCLUSION: This is the first study to establish a CAR NK cell line based on the human NK cell line KHYG-1. Therapy with EvCAR-KHYG-1 may be an effective treatment option for GBM patients.

# Inclusion of membrane-anchored LTB or flagellin protein in H5N1 virus-like particles enhances protective responses following intramuscular and oral immunization of mice.

2018

Vaccine

Ren, Zhiguang

Zhao, Yongkun

Liu, Jing

Ji, Xianliang

Meng, Lingnan

Wang, Tiecheng

Sun, Weiyang

Zhang, Kun

Sang, Xiaoyu

Yu, Zhijun

Li, Yuanguo

Feng, Na

Wang, Hualei

Yang, Songtao

Yang, Zhengyan

Wang, Zhizeng

Gao, Yuwei

Xia, Xianzhu

We previously demonstrated that intramuscular immunization with virus-like particles (VLPs) composed of the haemagglutinin (HA), neuraminidase (NA), and matrix (M1) proteins of A/meerkat/Shanghai/SH-1/2012 (clade 2.3.2.1) protected mice from lethal challenge with viruses from other H5 HPAI clades. The inclusion of additional proteins that can serve as immunological adjuvants in VLPs may enhance adaptive immune responses following vaccination, and oral vaccines may represent the safest choice. Here, we report the generation of H5N1 VLPs composed of the viral HA, NA, and M1 proteins and membrane-anchored forms of the Escherichia coli heat-labile enterotoxin B subunit protein (LTB) or the Toll-like receptor 5 ligand flagellin (Flic). Mice intramuscularly or orally immunized with VLPs containing LTB or Flic generated greater humoural and cellular immune responses than those administered H5N1 VLPs without LTB or Flic. Intramuscular immunization with VLPs protected mice from lethal challenge with homologous or heterologous H5N1 viruses irrespective of whether the VLPs additionally included LTB or Flic. In contrast, oral immunization of mice with LTB- or Flic-VLPs conferred substantial protection against lethal challenge with both homologous and heterologous H5N1 influenza viruses, whereas mice immunized orally with VLPs lacking LTB and Flic universally succumbed to infection. Mice immunized orally with LTB- or Flic-VLPs showed 10-fold higher virus-specific IgG titres than mice immunized with H5N1-VLPs lacking LTB or Flic. Collectively, these results indicate that the inclusion of immunostimulatory proteins, such as LTB and Flic, in VLP-based vaccines may represent a promising new approach for the control of current H5N1 HPAI outbreaks by eliciting higher humoural and cellular immune responses and conferring improved cross-clade protection.

# The hepatoprotective effects of Radix Bupleuri extracts against D-galactosamine/lipopolysaccharide induced liver injury in hybrid grouper (Epinephelus lanceolatusmale symbol x Epinephelus fuscoguttatusfemale symbol).

2018

Fish & shellfish immunology

Zou, Cuiyun

Tan, Xiaohong

Ye, Huaqun

Sun, Zhenzhu

Chen, Shu

Liu, Qingying

Xu, Minglei

Ye, Chaoxia

Wang, Anli

The present study is aiming at evaluating the hepatoprotective of Radix Bupleuri extracts (RBE) on the d-galactosamine/lipopolysaccharide (D-GalN/LPS) induced liver injury of hybrid grouper in vitro and in vivo. In vitro, RBE (0, 200, 400 and 800mug/ml) was added to the hybrid grouper primary hepatocytes before (pretreatment) the incubation of the hepatocytes with D-GalN (20mM) plus LPS (1mug/ml) in the culture medium. RBE at concentrations of 200, 400 and 800mug/ml significantly improved cell viability and inhibited the elevation of TNF-alpha, IL-1beta and IL-6 and significantly down-regulated the caspase-3, caspase-9 and P53 mRNA levels. In vivo administration of RBE at the doses of 0, 200, 400, 800 and 1600mg/kg in the diet for 8 weeks prior to D-GalN (500mg/kg) and LPS (20mug/kg) intoxication. The study indicated that the RBE not only ameliorated liver injury, as evidenced by well-preserved liver architecture, but also significantly increased hepatic antioxidant enzymes activities in the D-GalN/LPS-induced liver injury animal model. Further demonstrating the protective effects of the RBE, we found that pretreatment with the RBE up-regulated the expression of antioxidant genes (GPx and MnSOD), while down-regulated apoptosis-related genes (caspase-3, caspase-9 and P53), immune related genes (MHC2 and TLR3) and pro-inflammatory cytokines (TOR and IKKalpha) mRNA expression in the liver of hybrid grouper. In brief, the present study showed that RBE can protect hepatocyte injury induced by D-GalN/LPS through elevating antioxidant enzyme activity and suppressing apoptosis and immune inflammatory responses. The results support the use of RBE as a hepatoprotective in fish.

# The expression of LINE1-MET chimeric transcript identifies a subgroup of aggressive breast cancers.

2018

International journal of cancer

Miglio, Umberto

Berrino, Enrico

Panero, Mara

Ferrero, Giulio

Coscujuela Tarrero, Lucia

Miano, Valentina

Dell'Aglio, Carmine

Sarotto, Ivana

Annaratone, Laura

Marchio, Caterina

Comoglio, Paolo M

De Bortoli, Michele

Pasini, Barbara

Venesio, Tiziana

Sapino, Anna

Demethylation of the long interspersed nuclear element (LINE-1; L1) antisense promoter can result in transcription of neighboring sequences as for the L1-MET transcript produced by the L1 placed in the second intron of MET. To define the role of L1-MET, we investigated the sequence and the transcription of L1-MET in vitro models and heterogeneous breast cancers, previously reported to show other L1-derived transcripts. L1-MET expressing cell lines were initially identified in silico and investigated for L1-MET promoter methylation, cDNA sequence and cell fraction mRNA. The transcriptional level of L1-MET and MET were then evaluated in breast specimens, including 9 cancer cell lines, 41 carcinomas of different subtypes, and 11 normal tissues. In addition to a L1-MET transcript ending at MET exon 21, six novel L1-MET splice variants were identified. Normal breast tissues were negative for the L1-MET expression, whereas the triple-negative breast cancer (TNBC) and the high-grade carcinomas were enriched with the L1-MET mRNA (p = 0.005 and p = 0.018, respectively). In cancer cells and tissues the L1-MET expression was associated with its promoter hypomethylation (rho = -0.8 and -0.9, respectively). No correlation was found between L1-MET and MET mRNA although L1-MET expressing tumors with higher L1-MET/MET ratio were negative for the MET protein expression (p = 0.006). Besides providing the first identification and detailed description of L1-MET in breast cancer, we clearly demonstrate that higher levels of this transcript specifically recognize a subset of more aggressive carcinomas, mainly TNBC. We suggest the possible evaluation of L1-MET in the challenging diagnosis of early TNBCs.

# Chimeric GII.3/GII.6 norovirus capsid (VP1) proteins: characterization by electron microscopy, trypsin sensitivity and binding to histo-blood group antigens.

2018

Archives of virology

Ma, Shuhuan

Zheng, Lijun

Liu, Jinjin

Wang, Wenhui

Ma, Jie

Cheng, Xuhui

Ge, Lili

Wang, Mingchen

Huo, Yuqi

Shen, Shuo

GII.3 and GII.6 noroviruses (NoVs) are similar in several aspects, including the presence of a short sequence insertion in the P2 domain of the major capsid protein (VP1) and trypsin susceptibility of VP1-containing virus-like particles (VLPs). In this study, we generated two constructs with the S or P domains of VP1 from GII.3 and GII.6 NoV strains exchanged (GII.3S/GII.6P and GII.6S/GII.3P), and the resultant chimeric capsid proteins were expressed from recombinant baculoviruses. The assembly of VLPs was confirmed by electron microscopy, and the susceptibility of assembled VLPs to trypsin digestion was analyzed by SDS-PAGE. Salivary histo-blood group antigen (HBGA) binding and binding blockade assays were performed to determine the binding characteristics of chimeric VP1-containing VLPs with and without trypsin digestion. Our results indicated that both expressed GII.3S/GII.6P and GII.6S/GII.3P chimeric proteins successfully assembled into VLPs. Trypsin digestion of VLPs assembled from both chimeric proteins led to the generation of two fragments with molecular sizes similar to those of wild-type VP1-containing VLPs. An in vitro salivary HBGA binding assay demonstrated that VLPs assembled from both chimeric proteins exhibited enhanced binding after trypsin cleavage. An HBGA binding blockade assay indicated that the binding of GII.3S/GII.6P and GII.6S/GII.3P VLPs against salivary HBGAs could only be blocked by GII.3 and GII.6 NoV VLP-specific hyperimmune sera, respectively. For GII.6 and GII.3S/GII.6P VLPs, a difference in binding enhancement after trypsin cleavage was observed. Our results demonstrate that the S domains of GII.3 and GII.6 NoV VP1 are interchangeable and that the S domain affects the binding of the P domain to HBGAs.

# Contribution of Three Different Regions of Isocitrate Dehydrogenases from Psychrophilic and Psychrotolerant Bacteria to Their Thermal Properties.

2018

Current microbiology

Mouri, Yuka

Takada, Yasuhiro

Monomeric isocitrate dehydrogenases of a psychrophilic bacterium, Colwellia maris, and a psychrotolerant bacterium, Pseudomonas psychrophila, (CmIDH and PpIDH) are cold-adapted and mesophilic, respectively. On the other hand, previous studies revealed that the monomeric IDH of Azotobacter vinelandii (AvIDH) is also mesophilic and the regions 2 and 3 among three regions of this enzyme are involved in the thermal properties. Therefore, to examine whether the region(s) responsible for the mesophilic properties are common between PpIDH and AvIDH, the genes of chimeric IDHs exchanging three regions of PpIDH and CmIDH in various combinations were constructed and overexpressed as His-tagged recombinant proteins in the Escherichia coli cells, and the chimeric and wild-type PpIDH and CmIDH were purified with Ni-chelating affinity column chromatography. The swapping chimeras of the regions 2 or 3 in PpIDH and CmIDH showed lower and higher optimum temperatures for activities and their thermostabilities than the wild-type ones, respectively. On the other hand, the exchange of the respective region 1 hardly influenced these properties of the two IDHs. Therefore, the regions 2 and 3 of the two IDHs were confirmed to be involved in their thermal properties. These results were coincident with those of the previous study on chimeric IDHs between AvIDH and CmIDH, indicating that the common regions of AvIDH and PpIDH are responsible for their mesophilic properties and the amino acid residues involved in their thermal properties are present in the regions 2 and 3.

# Type C/D botulism in the waterfowl in an urban park in Italy.

2018

Anaerobe

Badagliacca, Pietro

Pomilio, Francesco

Auricchio, Bruna

Sperandii, Anna Franca

Di Provvido, Andrea

Di Ventura, Mauro

Migliorati, Giacomo

Caudullo, Mario

Morelli, Daniela

Anniballi, Fabrizio

This report describes an outbreak of botulism occurred among a free-living population of mallards (Anas platyrhynchos) and geese (Anser anser) in an urban park. Mortality rate among investigated population was 86,8% (118 dead out of 136). Twenty-seven carcasses were collected for macroscopic examination and screened for microbiological, virological, toxicological investigations. A sick mallard was captured and neurological symptoms were observed. No causative agent of viral avian diseases was found in the examined animals and screening for environmental neurotoxic substances proved negative as well. In contrast, microbiological cultures from specimens tested positive for botulinum toxin-producing clostridia. Blood serum and fecal extract of the sick mallard proved positive for botulinum neurotoxin in the standard mouse protection test using reference Clostridium botulinum type C antitoxin. Gene content of cultured strains showed a mosaic composition of bont/C and bont/D sequences, defining them as type C/D chimeric organisms.

# A YopH PTP1B Chimera Shows the Importance of the WPD-Loop Sequence to the Activity, Structure, and Dynamics of Protein Tyrosine Phosphatases.

2018

Biochemistry

Moise, Gwendolyn

Morales, Yalemi

Beaumont, Victor

Caradonna, Timothy

Loria, J Patrick

Johnson, Sean J

Hengge, Alvan C

To study factors that affect WPD-loop motion in protein tyrosine phosphatases (PTPs), a chimera of PTP1B and YopH was created by transposing the WPD loop from PTP1B to YopH. Several subsequent mutations proved to be necessary to obtain a soluble, active enzyme. That chimera, termed chimera 3, retains productive WPD-loop motions and general acid catalysis with a pH dependency similar to that of the native enzymes. Kinetic isotope effects show the mechanism and transition state for phosphoryl transfer are unaltered. Catalysis of the chimera is slower than that of either of its parent enzymes, although its rate is comparable to those of most native PTPs. X-ray crystallography and nuclear magnetic resonance were used to probe the structure and dynamics of chimera 3. The chimera's structure was found to sample an unproductive hyper-open conformation of its WPD loop, a geometry that has not been observed in either of the parents or in other native PTPs. The reduced catalytic rate is attributed to the protein's sampling of this conformation in solution, reducing the fraction in the catalytically productive loop-closed conformation.

# Influence of inter-domain dynamics and surrounding environment flexibility on the direct electrochemistry and electrocatalysis of self-sufficient cytochrome P450 3A4-BMR chimeras.

2018

Journal of inorganic biochemistry

Castrignano, Silvia

Di Nardo, Giovanna

Sadeghi, Sheila J

Gilardi, Gianfranco

The linker region of multi-domain enzymes has a very important role for the interconnection of different enzyme modules and for the efficiency of catalytic activity. This is particularly evident for artificial chimeric systems. We characterised an artificial self-sufficient enzyme developed by genetic fusion of the catalytic domain of cytochrome P450 3A4 and reductase domain of Bacillus megaterium BM3 (BMR). Here we report the direct electrochemistry of 3A4-BMR chimeras immobilised on glassy carbon electrodes and we investigated the effect of inter-domain loop length and immobilising environment flexibility on both redox properties and electrocatalysis. We observe that redox potential can be modulated by the linker length and the immobilising layer flexibility. In addition, enzyme inter-domain dynamics and environment flexibility also modulate 3A4-BMR turnover efficiency on electrode system. Vmax values are increased up to about 100% in the presence of testosterone and up to about 50% in presence of tamoxifen by decreasing immobilising film rigidity. The effect on 3A4-BMR Vmax values is dependent on inter-domain loop length with 3A4-5GLY-BMR chimera being the more affected. The underlying reason for these observations is the potential motion of the FMN domain that is the key to shuttle electrons from FAD to haem.

# Construction, Expression, and Characterization of a Novel Human-Mouse Chimeric Antibody, Hm3A4: A Potential Therapeutic Agent for B and Myeloid Lineage Leukemias.

2018

DNA and cell biology

Li, Sisi

Shen, Diying

Guo, Xiaoping

Liao, Chan

Tang, Yongmin

Antibody-targeting therapy has drawn great interests to the hematologists and oncologists. 3A4, a novel antibody recognizing human CD45RA antigen, is a new target molecule for leukemias and holds a therapeutic potential for myeloid lineage leukemias. However, murine antibodies cannot be safely used in patients because of their strong immune reaction, humanization of the antibodies interested will be an important development step for therapeutic purpose. The aim of this study was to engineer the mouse 3A4 and to investigate the biological activity of its chimeric form. The humanized antibody composed of the 3A4 single-chain fragment of variable region and the human IgG1 Fc region, which was named human-mouse chimeric antibody 3A4 (Hm3A4). The function and biological activities of Hm3A4 were characterized using a variety of biological approaches. The results showed that Hm3A4 retained a strong binding activity to its antigen and could significantly block the binding of parental 3A4 to the antigen. In vitro experiments revealed that Hm3A4 could kill the target cells through complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity function. In vivo, Hm3A4 showed efficient antileukemia activity outperforming the nontreated mice. In conclusion, the chimeric antibody has an excellent biological activity after humanization and holds targeting therapeutic potential for myeloid leukemia, which warrants further development of this agent.

# P450-Humanized and Human Liver Chimeric Mouse Models for Studying Xenobiotic Metabolism and Toxicity.

2018

Drug metabolism and disposition: the biological fate of chemicals

Bissig, Karl-Dimiter

Han, Weiguo

Barzi, Mercedes

Kovalchuk, Nataliia

Ding, Liang

Fan, Xiaoyu

Pankowicz, Francis P

Zhang, Qing-Yu

Ding, Xinxin

Preclinical evaluation of drug candidates in experimental animal models is an essential step in drug development. Humanized mouse models have emerged as a promising alternative to traditional animal models. The purpose of this mini-review is to provide a brief survey of currently available mouse models for studying human xenobiotic metabolism. Here, we describe both genetic humanization and human liver chimeric mouse models, focusing on the advantages and limitations while outlining their key features and applications. Although this field of biomedical science is relatively young, these humanized mouse models have the potential to transform preclinical drug testing and eventually lead to a more cost-effective and rapid development of new therapies.

# Human relevance of rodent liver tumour formation by constitutive androstane receptor (CAR) activators.

2018

Toxicology research

Lake, Brian G

A large number of nongenotoxic chemicals have been shown to increase the incidence of liver tumours in rats and/or mice by a mode of action (MOA) involving activation of the constitutive androstane receptor (CAR). Studies with the model CAR activator phenobarbital (PB) and its sodium salt (sodium phenobarbital; NaPB) have demonstrated that the key and associative events for rat and mouse liver tumour formation include CAR activation, increased hepatocyte replicative DNA synthesis (RDS), induction of cytochrome P450 CYP2B subfamily enzymes, liver hypertrophy, increased altered hepatic foci and hepatocellular adenomas/carcinomas. The key species difference between the rat and mouse compared to humans, is that human hepatocytes are refractory to the mitogenic effects of PB/NaPB and other CAR activators. While PB/NaPB and other CAR activators stimulate RDS in rat and mouse hepatocytes in both in vitro and in vivo studies, such compounds do not stimulate RDS in cultured human hepatocytes and in in vivo studies performed in chimeric mice with humanised livers. In terms of species differences in RDS, unlike the rat and mouse, humans are similar to other species such as the Syrian hamster and guinea pig in being nonresponsive to the mitogenic effects of CAR activators. Overall, the MOA for rat and mouse liver tumour formation by PB/NaPB and other CAR activators is considered qualitatively not plausible for humans. This conclusion is supported by data from a number of epidemiological studies, which demonstrate that chronic treatment with PB does not increase the incidence of liver cancer in humans.

# Chimeric analysis with newly established EGFP/DsRed2-tagged ES cells identify HYDIN as essential for spermiogenesis in mice.

2018

Experimental animals

Oura, Seiya

Miyata, Haruhiko

Noda, Taichi

Shimada, Keisuke

Matsumura, Takafumi

Morohoshi, Akane

Isotani, Ayako

Ikawa, Masahito

The CRISPR/Cas9 system can efficiently introduce biallelic mutations in ES cells (ESCs), and its application with fluorescently-tagged ESCs enables phenotype analysis in chimeric mice. We have utilized ESCs that express EGFP in the cytosol and acrosome [EGR-G101 129S2 x (CAG/Acr-EGFP) B6] in previous studies; however, the EGFP signal in the sperm cytosol is weak and the signal in the acrosome is lost after the acrosome reaction, precluding analysis between wild type and ESC derived spermatozoa. In this study, we established an ESC line from RBGS (Red Body Green Sperm) transgenic mice [B6D2-Tg (CAG/Su9-DsRed2, Acr3-EGFP) RBGS002Osb] whose spermatozoa exhibit green fluorescence in the acrosome and red fluorescence in the mitochondria within the flagellar midpiece that is retained after the acrosome reaction. We utilized these new ESCs to analyze HYDIN, which is reported to function in sperm motility in humans. Analysis of Hydin-disrupted spermatozoa in mice is difficult as Hydin-mutant mice (hy3) die within 3 weeks, before sexual maturation, due to hydrocephaly. To circumvent the early lethality of the whole-body knockout, we disrupted Hydin in RBGS-ESCs and generated chimeric mice, which survived into sexual maturity. Hydin-disrupted spermatozoa obtained from the chimeric mice possessed short tails and were immotile. When we injected Hydin-disrupted spermatozoa into oocytes, heterozygous pups were obtained, which suggests that the genome of Hydin-disrupted spermatozoa can produce viable pups. Consequently, RBGS-ESCs can be a useful tool for screening and analysis of male-fertility related genes in chimeric mice.

# A long-term culture system based on a collagen vitrigel membrane chamber that supports liver-specific functions of hepatocytes isolated from mice with humanized livers.

2018

The Journal of toxicological sciences

Watari, Ryuji

Kakiki, Motoharu

Oshikata, Ayumi

Takezawa, Toshiaki

Yamasaki, Chihiro

Ishida, Yuji

Tateno, Chise

Kuroda, Yukie

Ishida, Seiichi

Kusano, Kazutomi

During drug discovery, in vitro models are used to predict the in vivo pharmacokinetic and toxicological properties of drug candidates in humans. However, the conventional method of culturing human hepatocytes as monolayers does not necessarily replicate biologic reactions and does not support liver-specific functions, such as cytochrome P450 (CYP) activities, for prolonged periods. To remedy these problems and thus increase and prolong hepatic functions, we developed a culture system comprising a collagen vitrigel membrane (CVM) chamber and PXB-cells(R), fresh hepatocytes isolated from liver-humanized chimeric mice (PXB-mice(R)). To quantitatively assess our new system, we evaluated the activities of 5 major CYP isoforms (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A), albumin secretion, and urea synthesis. First, between Days 14 and 21, the activities of all CYP isoforms tested in vitrigel culture were equal to or higher than in conventional monolayer culture system. Second, the activities of CYP3A, CYP2C9, and CYP2C19 during Days 10 through 17 were higher in vitrigel culture than in suspended PXB-cells prepared on Day 0 (suspension assay). Third, albumin secretion and urea synthesis were higher in vitrigel culture than in conventional monolayer culture. Fourth, the vitrigel-cultured PXB-cells showed the characteristic morphology of parenchymal hepatocytes and were almost all alive in monolayer. These results indicate that our vitrigel culture method is superior to the conventional monolayer method in terms of diverse liver-specific functions, including CYP activity. Our findings suggest that the vitrigel culture method could be a powerful in vitro tool for predicting the pharmacokinetic and toxicological properties of drug candidates in humans.

# The chimeric TAC receptor co-opts the T cell receptor yielding robust anti-tumor activity without toxicity.

2018

Nature communications

Helsen, Christopher W

Hammill, Joanne A

Lau, Vivian W C

Mwawasi, Kenneth A

Afsahi, Arya

Bezverbnaya, Ksenia

Newhook, Lisa

Hayes, Danielle L

Aarts, Craig

Bojovic, Bojana

Denisova, Galina F

Kwiecien, Jacek M

Brain, Ian

Derocher, Heather

Milne, Katy

Nelson, Brad H

Bramson, Jonathan L

Engineering T cells with chimeric antigen receptors (CARs) is an effective method for directing T cells to attack tumors, but may cause adverse side effects such as the potentially lethal cytokine release syndrome. Here the authors show that the T cell antigen coupler (TAC), a chimeric receptor that co-opts the endogenous TCR, induces more efficient anti-tumor responses and reduced toxicity when compared with past-generation CARs. TAC-engineered T cells induce robust and antigen-specific cytokine production and cytotoxicity in vitro, and strong anti-tumor activity in a variety of xenograft models including solid and liquid tumors. In a solid tumor model, TAC-T cells outperform CD28-based CAR-T cells with increased anti-tumor efficacy, reduced toxicity, and faster tumor infiltration. Intratumoral TAC-T cells are enriched for Ki-67(+) CD8(+) T cells, demonstrating local expansion. These results indicate that TAC-T cells may have a superior therapeutic index relative to CAR-T cells.

# Reporter bacteriophage T7NLC utilizes a novel NanoLuc::CBM fusion for the ultrasensitive detection of Escherichia coli in water.

2018

The Analyst

Hinkley, T C

Garing, S

Singh, S

Le Ny, A-L M

Nichols, K P

Peters, J E

Talbert, J N

Nugen, S R

Rapid detection of bacteria responsible for foodborne diseases is a growing necessity for public health. Reporter bacteriophages (phages) are robust biorecognition elements uniquely suited for the rapid and sensitive detection of bacterial species. The advantages of phages include their host specificity, ability to distinguish viable and non-viable cells, low cost, and ease of genetic engineering. Upon infection with reporter phages, target bacteria express reporter enzymes encoded within the phage genome. In this study, the T7 coliphage was genetically engineered to express the newly developed luceriferase, NanoLuc (NLuc), as an indicator of bacterial contamination. While several genetic approaches were employed to optimize reporter enzyme expression, the novel achievement of this work was the successful fusion of the NanoLuc reporter to a carbohydrate binding module (CBM) with specificity to crystalline cellulose. This novel chimeric reporter (nluc::cbm) bestows the specific and irreversible immobilization of NanoLuc onto a low-cost, widely available crystalline cellulosic substrate. We have shown the possibility of detecting the immobilized fusion protein in a filter plate which resulted from a single CFU of E. coli. We then demonstrated that microcrystalline cellulose can be used to concentrate the fusion reporter from 100 mL water samples allowing a limit of detection of <10 CFU mL-1E. coli in 3 hours. Therefore, we conclude that our phage-based detection assay displays significant aptitude as a proof-of-concept drinking water diagnostic assay for the low-cost, rapid and sensitive detection of E. coli. Additional improvements in the capture efficiency of the phage-based fusion reporter should allow a limit of detection of <10 CFU per 100 mL.

# Targeting the IDO1 pathway in cancer: from bench to bedside.

2018

Journal of hematology & oncology

Liu, Ming

Wang, Xu

Wang, Lei

Ma, Xiaodong

Gong, Zhaojian

Zhang, Shanshan

Li, Yong

Indoleamine 2, 3-dioxygenases (IDO1 and IDO2) and tryptophan 2, 3-dioxygenase (TDO) are tryptophan catabolic enzymes that catalyze the conversion of tryptophan into kynurenine. The depletion of tryptophan and the increase in kynurenine exert important immunosuppressive functions by activating T regulatory cells and myeloid-derived suppressor cells, suppressing the functions of effector T and natural killer cells, and promoting neovascularization of solid tumors. Targeting IDO1 represents a therapeutic opportunity in cancer immunotherapy beyond checkpoint blockade or adoptive transfer of chimeric antigen receptor T cells. In this review, we discuss the function of the IDO1 pathway in tumor progression and immune surveillance. We highlight recent preclinical and clinical progress in targeting the IDO1 pathway in cancer therapeutics, including peptide vaccines, expression inhibitors, enzymatic inhibitors, and effector inhibitors.

# [Enhancing thermal stability of glucose oxidase by fusing amphiphilic short peptide].

2018

Sheng wu gong cheng xue bao = Chinese journal of biotechnology

Ren, Chunhui

Zhang, Juan

Du, Guocheng

Chen, Jian

Glucose oxidase catalyzes the oxidation of beta-D-glucose to gluconic acid and its derivatives, thus shows a great potential in the development of antibiotic-free feed. However, its production and processing still have the problem of poor thermal stability of enzyme activity. In this study, fusion of amphiphilic peptide technology was used to improve the stability of glucose oxidase. Herein, eight self-assembling peptides with different amino acid lengths and Linkers were fused to the N terminus of the glucose oxidase, yielding eight chimeric fusions SAP1-GS-GOD, SAP1-PT-GOD, SAP2-PT-GOD, SAP3-PT-GOD, SAP4-PT-GOD, SAP5-PT-GOD, SAP6-PT-GOD and SAP7-PT-GOD. Then, the 8 recombinant proteins were expressed in P. pastoris GS115. After separation and purification, the stability of glucose oxidase at 60 was determined. The relative enzyme activities of the PT Linker-linked fusion enzyme incubated at 60 for 60 min were higher than those of the original enzyme, and the relative activity of SAP5-PT-GOD was 67% at 60 for 30 min, which was 10.9 times higher than that of the initial enzyme with the same treatment. Among them, the Kcat/Km value of SAP1-PT-GOD, SAP2-PT-GOD, SAP3-PT-GOD and SAP5-PT-GOD of the fusion enzyme was further improved than that of the initial enzyme. Through the analysis of the intramolecular force of the fusion enzyme, the increase of the thermal stability of the fusion enzyme is mainly due to the increase of the hydrogen bond. In summary, the study indicates that translational fusion of self-assembling peptides with PT Linker was able to augment the thermo-stability of glucose oxidase, which has certain potential in the production and application of glucose oxidase. The glucose oxidase with improved thermostability obtained in the above study and the related mechanism will play an important role in improving the activity of related enzymes in the proceeding of processing and application.

# Development of monoclonal anti-PDGF-CC antibodies as tools for investigating human tissue expression and for blocking PDGF-CC induced PDGFRalpha signalling in vivo.

2018

PloS one

Li, Hong

Zeitelhofer, Manuel

Nilsson, Ingrid

Liu, Xicong

Allan, Laura

Gloria, Benjamin

Perani, Angelo

Murone, Carmel

Catimel, Bruno

Neville, A Munro

Scott, Fiona E

Scott, Andrew M

Eriksson, Ulf

PDGF-CC is a member of the platelet-derived growth factor (PDGF) family that stimulates PDGFRalpha phosphorylation and thereby activates intracellular signalling events essential for development but also in cancer, fibrosis and neuropathologies involving blood-brain barrier (BBB) disruption. In order to elucidate the biological and pathological role(s) of PDGF-CC signalling, we have generated high affinity neutralizing monoclonal antibodies (mAbs) recognizing human PDGF-CC. We determined the complementarity determining regions (CDRs) of the selected clones, and mapped the binding epitope for clone 6B3. Using the monoclonal 6B3, we determined the expression pattern for PDGF-CC in different human primary tumours and control tissues, and explored its ability to neutralize PDGF-CC-induced phosphorylation of PDGFRalpha. In addition, we showed that PDGF-CC induced disruption of the blood-retinal barrier (BRB) was significantly reduced upon intraperitoneal administration of a chimeric anti-PDGF-CC antibody. In summary, we report on high affinity monoclonal antibodies against PDGF-CC that have therapeutic efficacy in vivo.

# SWATH-MS based quantitative proteomics analysis reveals that curcumin alters the metabolic enzyme profile of CML cells by affecting the activity of miR-22/IPO7/HIF-1alpha axis.

2018

Journal of experimental & clinical cancer research : CR

Monteleone, Francesca

Taverna, Simona

Alessandro, Riccardo

Fontana, Simona

BACKGROUND: Chronic myelogenous leukemia (CML) is a myeloproliferative disorder caused by expression of the chimeric BCR-ABL tyrosine kinase oncogene, resulting from the t(9;22) chromosomal translocation. Imatinib (gleevec, STI-571) is a selective inhibitor of BCR-ABL activity highly effective in the treatment of CML. However, even though almost all CML patients respond to treatment with imatinib or third generation inhibitors, these drugs are not curative and need to be taken indefinitely or until patients become resistant. Therefore, to get a definitive eradication of leukemic cells, it is necessary to find novel therapeutic combinations, for achieving greater efficacy and fewer side effects. Curcumin is an Indian spice with several therapeutic properties: anti-oxidant, analgesic, anti-inflammatory, antiseptic and anti-cancer. In cancer disease, it acts by blocking cell transformation, proliferation, and invasion and by inducing cell apoptosis. METHODS: In the present study, the effect of a sub-toxic dose of curcumin on K562 cells was evaluated by using the technique of Sequential Window Activation of All Theoretical Mass Spectra (SWATH-MS). Bioinformatic analysis of proteomic data was performed to highlight the pathways mostly affected by the treatment. The involvement of Hypoxia inducible factor 1 alpha (HIF-1alpha) was assayed by evaluating its activation status and the modulation of importin 7 (IPO7) and miR-22 was assessed by quantitative PCR and western blot analysis. Finally, K562 cells transfected with miR-22 inhibitor were used to confirm the ability of curcumin to elicit miR-22 expression. RESULTS: Our findings revealed that the most relevant effect induced by curcumin was a consistent decrease of several proteins involved in glucose metabolism, most of which were HIF-1alpha targets, concomitant with the up-regulation of functional and structural mitochondrial proteins. The mechanism by which curcumin affects metabolic enzyme profile was associated with the reduction of HIF-1alpha activity, due to the miR-22-mediated down-regulation of IPO7 expression. Finally, the ability of curcumin to enhance in vitro the efficiency of imatinib was reported. CONCLUSIONS: In summary, our data indicates that the miR-22/IPO7/HIF-1alpha axis may be considered as a novel molecular target of curcumin adding new insights to better define therapeutic activity and anticancer properties of this natural compound. The MS proteomic data have been deposited to the ProteomeXchange with identifier <PXD007771>.

# Activity of the Chimeric Lysin ClyR against Common Gram-Positive Oral Microbes and Its Anticaries Efficacy in Rat Models.

2018

Viruses

Xu, Jingjing

Yang, Hang

Bi, Yongli

Li, Wuyou

Wei, Hongping

Li, Yuhong

Dental caries is a common disease caused by oral bacteria. Streptococcus mutans and Streptococcus sobrinus are the primary cariogenic microbes that often survive as biofilms on teeth. In this study, we evaluated the activity of ClyR, a well-known chimeric lysin with extended streptococcal host range, against common Gram-positive oral microbes and its anticaries efficacy in rat models. ClyR demonstrated high lytic activity against S. mutans MT8148 and S. sobrinus ATCC6715, with minor activity against Streptococcus sanguinis, Streptococcus oralis, and Streptococcus salivarius, which are considered as harmless commensal oral bacteria. Confocal laser scanning microscopy showed that the number of viable cells in 72-h aged S. mutans and S. sobrinus biofilms are significantly (p < 0.05) decreased after treatment with 50 microg/mL ClyR for 5 min. Furthermore, continuous administration of ClyR for 40 days (5 microg/day) significantly (p < 0.05) reduced the severity of caries in rat models infected with a single or a mixed bacteria of S. mutans and S. sobrinus. Therefore, ClyR could be a promising agent or additive for the prevention and treatment of dental caries.

# Development of GFP-based high-throughput screening system for directed evolution of glucose oxidase.

2018

Journal of bioscience and bioengineering

Kovacevic, Gordana

Ostafe, Raluca

Balaz, Ana Marija

Fischer, Rainer

Prodanovic, Radivoje

Glucose oxidase (GOx) mutants with higher activity or stability have important role in industry and in the development of biosensors and biofuel cells. Discovering these mutants can be time-consuming if appropriate high-throughput screening (HTS) systems are not available. GOx gene libraries were successfully screened and sorted using a HTS system based on GOx activity dependent fluorescent labeling of yeast cells with tyramids and quantification of the amount of expressed enzyme by yeast enhanced green fluorescent protein (yGFP) tagging and flow cytometry. For this purpose, we expressed wild type and a mutant GOx as a chimera with the yGFP to confirm differences in catalytic activity between wild-type and mutant GOx. Fluorescence of yGFP is preserved during expression of chimera, and also after the oxidative enzymatic reaction. We have obtained a 2.5-fold enrichment in population of cells expressing active enzyme, and percentage of enzyme variants with enzymatic mean activity higher than wild type activity was increased to 44% after a single round of GOx gene library sorting. We have found two mutants with 1.3 and 2.3-fold increase in Vmax values compared to the wtGOx. By simultaneous detection of protein expression level and enzyme activity we have increased the likelihood of finding GOx variants with increased activity in a single round of flow cytometry sorting.

# Exchange of functional domains between a bacterial conjugative relaxase and the integrase of the human adeno-associated virus.

2018

PloS one

Agundez, Leticia

Zarate-Perez, Francisco

Meier, Anita F

Bardelli, Martino

Llosa, Matxalen

Escalante, Carlos R

Linden, R Michael

Henckaerts, Els

Endonucleases of the HUH family are specialized in processing single-stranded DNA in a variety of evolutionarily highly conserved biological processes related to mobile genetic elements. They share a structurally defined catalytic domain for site-specific nicking and strand-transfer reactions, which is often linked to the activities of additional functional domains, contributing to their overall versatility. To assess if these HUH domains could be interchanged, we created a chimeric protein from two distantly related HUH endonucleases, containing the N-terminal HUH domain of the bacterial conjugative relaxase TrwC and the C-terminal DNA helicase domain of the human adeno-associated virus (AAV) replicase and site-specific integrase. The purified chimeric protein retained oligomerization properties and DNA helicase activities similar to Rep68, while its DNA binding specificity and cleaving-joining activity at oriT was similar to TrwC. Interestingly, the chimeric protein could catalyse site-specific integration in bacteria with an efficiency comparable to that of TrwC, while the HUH domain of TrwC alone was unable to catalyze this reaction, implying that the Rep68 C-terminal helicase domain is complementing the TrwC HUH domain to achieve site-specific integration into TrwC targets in bacteria. Our results illustrate how HUH domains could have acquired through evolution other domains in order to attain new roles, contributing to the functional flexibility observed in this protein superfamily.

# Oncogenic Function of a KIF5B-MET Fusion Variant in Non-Small Cell Lung Cancer.

2018

Neoplasia (New York, N.Y.)

Gow, Chien-Hung

Liu, Yi-Nan

Li, Huei-Ying

Hsieh, Min-Shu

Chang, Shih-Han

Luo, Sheng-Ching

Tsai, Tzu-Hsiu

Chen, Pei-Lung

Tsai, Meng-Feng

Shih, Jin-Yuan

A kinesin family member 5b (KIF5B)-MET proto-oncogene, receptor tyrosine kinase (MET) rearrangement was reported in patients with lung adenocarcinoma but its oncogenic function was not fully evaluated. We used one-step reverse transcription-polymerase chain reaction for RNA samples to screen for the KIF5B-MET fusion in 206 lung adenocarcinoma and 28 pulmonary sarcomatoid carcinoma patients. Genomic breakpoints of KIF5B-MET were determined by targeted next-generation sequencing. Soft agar colony formation assays, proliferation assays, and a xenograft mouse model were used to investigate its oncogenic activity. In addition, specific MET inhibitors were administered to evaluate their anti-tumor activities. A KIF5B-MET fusion variant in a patient with a mixed-type adenocarcinoma and sarcomatoid tumor was identified, and another case was found in a pulmonary sarcomatoid carcinoma patient. Both cases carried the same chimeric gene, a fusion between exons 1-24 of KIF5B and exons 15-21 of MET. KIF5B-MET-overexpressing cells exhibited significantly increased proliferation and colony-forming ability. Xenograft tumors harboring the fusion gene demonstrated significantly elevated tumor growth. Ectopic expression of the fusion gene stimulated the phosphorylation of KIF5B-MET as well as downstream STAT3, AKT, and ERK1/2 signaling pathways. The MET inhibitors significantly repressed cell proliferation; phosphorylation of downstream STAT3, AKT, and ERK1/2; and xenograft tumorigenicity. In conclusion, the KIF5B-MET variant was demonstrated to have an oncogenic function in cancer cells. These findings have immediate clinical implications for the targeted therapy of subgroups of non-small cell lung cancer patients.

# Astrocytes restore connectivity and synchronization in dysfunctional cerebellar networks.

2018

Proceedings of the National Academy of Sciences of the United States of America

Kanner, Sivan

Goldin, Miri

Galron, Ronit

Ben Jacob, Eshel

Bonifazi, Paolo

Barzilai, Ari

Evidence suggests that astrocytes play key roles in structural and functional organization of neuronal circuits. To understand how astrocytes influence the physiopathology of cerebellar circuits, we cultured cells from cerebella of mice that lack the ATM gene. Mutations in ATM are causative of the human cerebellar degenerative disease ataxia-telangiectasia. Cerebellar cultures grown from Atm(-/-) mice had disrupted network synchronization, atrophied astrocytic arborizations, reduced autophagy levels, and higher numbers of synapses per neuron than wild-type cultures. Chimeric circuitries composed of wild-type astrocytes and Atm(-/-) neurons were indistinguishable from wild-type cultures. Adult cerebellar characterizations confirmed disrupted astrocyte morphology, increased GABAergic synaptic markers, and reduced autophagy in Atm(-/-) compared with wild-type mice. These results indicate that astrocytes can impact neuronal circuits at levels ranging from synaptic expression to global dynamics.

# [A DNA Construct That Encodes the Rabies Virus Consensus Glycoprotein with a Proteasome Degradation Signal Induces Antibody Production with IgG2A Subtype Predominance].

2018

Molekuliarnaia biologiia

Starodubova, E S

Kuzmenko, Yu V

Pankova, E O

Latanova, A A

Preobrazhenskaya, O V

Karpov, V L

The possibility of enhancing the immunogenicity of the rabies virus glycoprotein antigen encoded by a DNA vaccine has been investigated. Ubiquitin-like protein FAT10 has been attached to the N-terminus of the glycoprotein to target it to the proteasome and stimulate its presentation by MHC class I. Two forms of the protein, chimeric and original, have been detected in cells transfected with the DNA construct encoding the chimeric protein. The presence of the glycoprotein on the cell surface has been detected by immunostaining of transfected cells. The production of IgG and IgG2a antibodies has been more efficiently induced in mice immunized with the plasmid that encodes the chimeric protein than in those immunized with the plas-mid that encodes unmodified glycoprotein. Moreover, the level of IgG2a antibodies exceeded the level of IgG1 antibodies, which indicates a preferential increase in the Th1 component of the immune response. The proposed DNA construct that encodes a modified glycoprotein with a proteasome degradation signal maybe a promising DNA vaccine immunogen for post-exposure prophylaxis of rabies.

# Characterization of Plasmodium vivax Proteins in Plasma-Derived Exosomes From Malaria-Infected Liver-Chimeric Humanized Mice.

2018

Frontiers in microbiology

Gualdron-Lopez, Melisa

Flannery, Erika L

Kangwanrangsan, Niwat

Chuenchob, Vorada

Fernandez-Orth, Dietmar

Segui-Barber, Joan

Royo, Felix

Falcon-Perez, Juan M

Fernandez-Becerra, Carmen

Lacerda, Marcus V G

Kappe, Stefan H I

Sattabongkot, Jetsumon

Gonzalez, Juan R

Mikolajczak, Sebastian A

Del Portillo, Hernando A

Exosomes are extracellular vesicles of endocytic origin containing molecular signatures implying the cell of origin; thus, they offer a unique opportunity to discover biomarkers of disease. Plasmodium vivax, responsible for more than half of all malaria cases outside Africa, is a major obstacle in the goal of malaria elimination due to the presence of dormant liver stages (hypnozoites), which after the initial infection may reactivate to cause disease. Hypnozoite infection is asymptomatic and there are currently no diagnostic tools to detect their presence. The human liver-chimeric (FRG huHep) mouse is a robust P. vivax infection model for exo-erythrocytic development of liver stages, including hypnozoites. We studied the proteome of plasma-derived exosomes isolated from P. vivax infected FRG huHep mice with the objective of identifying liver-stage expressed parasite proteins indicative of infection. Proteomic analysis of these exosomes showed the presence of 290 and 234 proteins from mouse and human origin, respectively, including canonical exosomal markers. Human proteins include proteins previously detected in liver-derived exosomes, highlighting the potential of this chimeric mouse model to study plasma exosomes derived unequivocally from human hepatocytes. Noticeably, we identified 17 parasite proteins including enzymes, surface proteins, components of the endocytic pathway and translation machinery, as well as uncharacterized proteins. Western blot analysis validated the presence of human arginase-I and an uncharacterized P. vivax protein in plasma-derived exosomes. This study represents a proof-of-principle that plasma-derived exosomes from P. vivax infected FRG-huHep mice contain human hepatocyte and P. vivax proteins with the potential to unveil biological features of liver infection and identify biomarkers of hypnozoite infection.

# A126 in the active site and TI167/168 in the TI loop are essential determinants of the substrate specificity of PTEN.

2018

Cellular and molecular life sciences : CMLS

Leitner, Michael G

Hobiger, Kirstin

Mavrantoni, Angeliki

Feuer, Anja

Oberwinkler, Johannes

Oliver, Dominik

Halaszovich, Christian R

PTEN prevents tumor genesis by antagonizing the PI3 kinase/Akt pathway through D3 site phosphatase activity toward PI(3,4)P2 and PI(3,4,5)P3. The structural determinants of this important specificity remain unknown. Interestingly, PTEN shares remarkable homology to voltage-sensitive phosphatases (VSPs) that dephosphorylate D5 and D3 sites of PI(4,5)P2, PI(3,4)P2, and PI(3,4,5)P3. Since the catalytic center of PTEN and VSPs differ markedly only in TI/gating loop and active site motif, we wondered whether these differences explained the variation of their substrate specificity. Therefore, we introduced mutations into PTEN to mimic corresponding sequences of VSPs and studied phosphatase activity in living cells utilizing engineered, voltage switchable PTENCiV, a Ci-VSP/PTEN chimera that retains D3 site activity of the native enzyme. Substrate specificity of this enzyme was analyzed with whole-cell patch clamp in combination with total internal reflection fluorescence microscopy and genetically encoded phosphoinositide sensors. In PTENCiV, mutating TI167/168 in the TI loop into the corresponding ET pair of VSPs induced VSP-like D5 phosphatase activity toward PI(3,4,5)P3, but not toward PI(4,5)P2. Combining TI/ET mutations with an A126G exchange in the active site removed major sequence variations between PTEN and VSPs and resulted in D5 activity toward PI(4,5)P2 and PI(3,4,5)P3 of PTENCiV. This PTEN mutant thus fully reproduced the substrate specificity of native VSPs. Importantly, the same combination of mutations also induced D5 activity toward PI(3,4,5)P3 in native PTEN demonstrating that the same residues determine the substrate specificity of the tumor suppressor in living cells. Reciprocal mutations in VSPs did not alter their substrate specificity, but reduced phosphatase activity. In summary, A126 in the active site and TI167/168 in the TI loop are essential determinants of PTEN's substrate specificity, whereas additional features might contribute to the enzymatic activity of VSPs.

# Usage of GD-95 and GD-66 lipases as fusion partners leading to improved chimeric enzyme LipGD95-GD66.

2018

International journal of biological macromolecules

Malunavicius, Vilius

Druteika, Gytis

Sadauskas, Mikas

Veteikyte, Ausra

Matijosyte, Inga

Lastauskiene, Egle

Gegeckas, Audrius

Gudiukaite, Renata

Lipases are used as biocatalysts in industrial processes mainly because of their stability at broad temperature and pH range, resistance to organic solvents and wide spectrum of substrates. The usage of several lipolytic domains, each with different activity and resistance profiles, enables both the flexibility and efficiency of industrial processes. In this study, GD-95 and GD-66 lipases produced by Geobacillus sp. 95 and Geobacillus sp. 66, respectively, were used as fusion partners to create a new fused lipolytic enzyme LipGD95-GD66. Chimeric LipGD95-GD66 lipase displayed tenfold increase in activity (200U/mg) compared to parental GD-66 lipase, improved Vmax (10mumol/minmg(-1)) and catalytic efficiency (2 \*10(5)min(-1)mM(-1)) for p-NP palmitate as a substrate and increased activity at 70-75 degrees C compared to both parental lipases. All three lipases also retained >50% of their lipolytic activity after incubation with methanol, n-hexane, ethanol and DMF for longer than three weeks, highlighting a great prospect for application in industrial processes. Moreover, transesterification results revealed the capability of parental GD-95 lipase to be the most promising biocatalyst for production of methyl and ethyl esters through eco-friendly transesterification using argan oil and ethanol/methanol as acceptors of acyl group.

# GSK3 inhibition, but not epigenetic remodeling, mediates efficient derivation of germline embryonic stem cells from nonobese diabetic mice.

2018

Stem cell research

Liu, Jun

Ashton, Michelle P

O'Bryan, Moira K

Brodnicki, Thomas C

Verma, Paul J

The nonobese diabetic (NOD) mouse strain is a predominant animal model of type 1 diabetes. However, this mouse strain is considered to be non-permissive for embryonic stem cell (ESC) derivation using conventional methods. We examined small molecule inhibition of glycogen synthase kinase 3 (GSK3) to block spontaneous cell differentiation and promote pluripotency persistence. Here we show a single pharmacological GSK3 inhibitor, 6-bromoindirubin-3'-oxime (BIO), in combination with leukemia inhibition factor (LIF), promoted generation of stable NOD ESC lines at >80% efficiency. Significantly, expansion of the established NOD ESC lines no longer required treatment with BIO. These NOD ESC lines contributed to chimeric mice and transmitted to germline progeny that spontaneously developed diabetes. By contrast, 5-aza-2'-deoxycytidine (AZA), a small molecule inhibitor of DNA methylation, and trichostatin A (TSA) and valproic acid (VPA), small molecule inhibitors of histone deacetylase, could not promote generation of NOD ESCs by epigenetic remodeling. These combined findings provide strategic insights for imposing pluripotency in cells isolated from a non-permissive strain.

# Development of third generation anti-EGFRvIII chimeric T cells and EGFRvIII-expressing artificial antigen presenting cells for adoptive cell therapy for glioma.

2018

PloS one

Sahin, Ayguen

Sanchez, Carlos

Bullain, Szofia

Waterman, Peter

Weissleder, Ralph

Carter, Bob S

Glioblastoma multiforme (GBM) is the most aggressive and deadly form of adult brain cancer. Despite of many attempts to identify potential therapies for this disease, including promising cancer immunotherapy approaches, it remains incurable. To address the need of improved persistence, expansion, and optimal antitumor activity of T-cells in the glioma milieu, we have developed an EGFRvIII-specific third generation (G3-EGFRvIII) chimeric antigen receptor (CAR) that expresses both co-stimulatory factors CD28 and OX40 (MR1-CD8TM-CD28-OX40-CD3zeta). To enhance ex vivo target specific activation and optimize T-cell culturing conditions, we generated artificial antigen presenting cell lines (aAPC) expressing the extracellular and transmembrane domain of EGFRvIII (EGFRVIIIDelta654) with costimulatory molecules including CD32, CD80 and 4-1BBL (EGFRVIIIDelta654 aAPC and CD32-80-137L-EGFRVIIIDelta654 aAPC). We demonstrate that the highest cell growth was achieved when G3-EGFRvIII CAR T-cells were cocultured with both co-stimulatory aAPCs and with exposure to EGFRvIII (CD32-80-137L-EGFRVIIIDelta654 aAPCs) in culturing periods of three to six weeks. G3-EGFRvIII CAR T-cells showed an increased level of IFN-gamma when cocultured with CD32-80-137L-EGFRVIIIDelta654 aAPCs. Evaluation of G3-EGFRvIII CAR T-cells in an orthotropic human glioma xenograft model demonstrated a prolonged survival of G3-EGFRvIII CAR treated mice compared to control mice. Importantly, we observed survival of G3-EGFRvIII CAR T-cells within the tumor as long as 90 days after implantation in low-dose and single administration, accompanied by a marked tumor stroma demolition. These findings suggest that G3-EGFRvIII CAR cocultured with CD32-80-137L-EGFRVIIIDelta654 aAPCs warrants itself as a potential anti-tumor therapy strategy for glioblastoma.

# The MocR-like transcription factors: pyridoxal 5'-phosphate-dependent regulators of bacterial metabolism.

2018

The FEBS journal

Tramonti, Angela

Nardella, Caterina

di Salvo, Martino L

Pascarella, Stefano

Contestabile, Roberto

Many biological functions played by current proteins were not created by evolution from scratch, rather they were obtained combining already available protein scaffolds. This is the case of MocR-like bacterial transcription factors (MocR-TFs), a subclass of GntR transcription regulators, whose structure is the outcome of the fusion between DNA-binding proteins and pyridoxal 5'-phosphate (PLP)-dependent enzymes. The resultant chimeras can count on the properties of both protein classes, i.e. the capability to recognize specific DNA sequences and to bind PLP and amino-compounds; it is the modulation of such binding properties to confer to MocR-TFs chimeras the ability to interact with effector molecules and DNA so as to regulate transcription. MocR-TFs control different metabolic processes involving vitamin B6 and amino acids, which are canonical ligands of PLP-dependent enzymes. However, MocR-TFs are also implicated in the metabolism of compounds that are not substrates of PLP-dependent enzymes, such as rhizopine and ectoine. Genomic analyses show that MocR-TFs are widespread among eubacteria, implying an essential role in their metabolism and highlighting the scarcity of our knowledge on these important players in microbial metabolism. Although MocR-TFs have been discovered 15 years ago, the research activity on these transcriptional regulators has only recently intensified, producing a wealth of information that needs to be brought back to general principles. This is the main task of this review, which reports and analyses the available information concerning MocR-TFs functional role, structural features, interaction with effector molecules and the characteristics of DNA transcriptional factor-binding sites of MocR-based regulatory systems.

# Expression and inducibility of cytochrome P450s in human hepatocytes isolated from chimeric mice with humanised livers.

2018

Xenobiotica; the fate of foreign compounds in biological systems

Uehara, Shotaro

Higuchi, Yuichiro

Yoneda, Nao

Yamazaki, Hiroshi

Suemizu, Hiroshi

The evaluation of drug-mediated cytochrome P450 (P450) induction using human hepatocytes is important for predicting drug interactions. In this study, we prepared hepatocytes from chimeric mice with humanised livers (Hu-Liver mice) and evaluated the expression and inducibility of P450s in these hepatocytes. Up to 95% of the Hu-Liver cells stained positive for human leukocyte antigen and the mean viability exceeded 85% (n = 10). Monolayer-cultured Hu-Liver cells displayed a similar morphology to cultures of the corresponding human hepatocytes used as transplantation donors. The mRNA expression levels in Hu-Liver cells of 16 P450 forms belonging to P450 subfamilies 1-4 correlated well with the expression levels of the same enzymes in human hepatocytes. The variations in individual P450 mRNA levels between Hu-Liver cells and the corresponding human hepatocytes were within five-fold for 13 P450 forms. The production of 6beta-hydroxytestosterone in Hu-Liver cells was significantly increased (p < .05) following treatment with the CYP3A inducer, rifampicin. Hu-Liver cells have characteristics similar to those of human hepatocytes in terms of mRNA expression levels and the inducibility of the various P450 forms. Thus, Hu-Liver cells can potentially be used for in vitro drug-mediated induction assays of human hepatic P450s.

# Uhrf1 regulates active transcriptional marks at bivalent domains in pluripotent stem cells through Setd1a.

2018

Nature communications

Kim, Kun-Yong

Tanaka, Yoshiaki

Su, Juan

Cakir, Bilal

Xiang, Yangfei

Patterson, Benjamin

Ding, Junjun

Jung, Yong-Wook

Kim, Ji-Hyun

Hysolli, Eriona

Lee, Haelim

Dajani, Rana

Kim, Jonghwan

Zhong, Mei

Lee, Jeong-Heon

Skalnik, David

Lim, Jeong Mook

Sullivan, Gareth J

Wang, Jianlong

Park, In-Hyun

Embryonic stem cells (ESCs) maintain pluripotency through unique epigenetic states. When ESCs commit to a specific lineage, epigenetic changes in histones and DNA accompany the transition to specialized cell types. Investigating how epigenetic regulation controls lineage specification is critical in order to generate the required cell types for clinical applications. Uhrf1 is a widely known hemi-methylated DNA-binding protein, playing a role in DNA methylation through the recruitment of Dnmt1 and in heterochromatin formation alongside G9a, Trim28, and HDACs. Although Uhrf1 is not essential in ESC self-renewal, it remains elusive how Uhrf1 regulates cell specification. Here we report that Uhrf1 forms a complex with the active trithorax group, the Setd1a/COMPASS complex, to maintain bivalent histone marks, particularly those associated with neuroectoderm and mesoderm specification. Overall, our data demonstrate that Uhrf1 safeguards proper differentiation via bivalent histone modifications.