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Absolute quantitation of MET using mass spectrometry for clinical application: assay precision, stability, and correlation with MET gene amplification in FFPE tumor tissue

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Background: Overexpression of Met tyrosine kinase receptor is associated with poor prognosis. Overexpression, and particularly *MET* amplification, are predictive of response to Met-specific therapy in preclinical models. Immunohistochemistry (IHC) of formalin-fixed paraffin-embedded (FFPE) tissues is currently used to select for 'high Met' expressing tumors for Met inhibitor trials. IHC suffers from antibody non-specificity, lack of quantitative resolution, and, when quantifying multiple proteins, inefficient use of scarce tissue.
Methods: After describing the development of the Liquid-Tissue-Selected Reaction Monitoring-mass spectrometry (LT-SRM-MS) Met assay, we evaluated the expression level of Met in 130 FFPE gastroesophageal cancer (GEC) tissues. We assessed the correlation of SRM Met expression to IHC and mean *MET* gene copy number (GCN)/nucleus or *MET*/*CEP7 ratio* by fluorescence in situ hybridization (FISH).
Results: Proteomic mapping of recombinant Met identified ^{418}TEFTTALQR^{426} as the optimal SRM peptide. Limits of detection (LOD) and quantitation (LOQ) for this peptide were 150 and 200 amol/μg tumor protein, respectively. The assay demonstrated excellent precision and temporal stability of measurements in serial sections analyzed one year apart. Expression levels of 130 GEC tissues ranged (<150 amol/μg to 4669.5 amol/μg. High correlation was observed between SRM Met expression and both *MET* GCN and *MET*/*CEP7* ratio as determined by FISH ($n = 30$; $R^2 = 0.898$). IHC did not correlate well with SRM ($n = 44$; $R^2 = 0.537$) nor FISH GCN ($n = 31$; $R^2 = 0.509$). A Met SRM level of $\geqslant$1500 amol/μg was 100% sensitive (95% CI 0.69−1) and 100% specific (95% CI 0.92−1) for *MET* amplification.
Conclusions: The Met SRM assay measured the absolute Met levels in clinical tissues with high precision. Compared to IHC, SRM provided a quantitative and linear measurement of Met expression, reliably distinguishing between non-amplified and amplified *MET* tumors. These results demonstrate a novel clinical tool for efficient tumor expression profiling, potentially leading to better informed therapeutic decisions for patients with GEC.

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Development of a novel anti-tumor antibody targeting CXADR

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Cancer chemotherapy using antibodies against cancer-associated molecules is one of promising approaches. In order to develop effective anti-tumor antibodies we have tried to find new molecular targets to raise antibodies. Previously we established highly tumorigenic subline LNCaP-CR cells from human prostate cancer LNCaP cells. Using both cell lines we assessed the signal sequence trap by retrovirus-meditated expression (SST-REX) method. SST-REX method could identify membranous and secreted proteins and revealed that LNCaP-CR cells highly expressed coxsackie virus and adenovirus receptor (CXADR) compared to the parental cells. We then constructed monoclonal antibodies against human CXADR and examined their anti-tumor effects. Among them, 6G10A clone antibody did not directly affect the growth of LNCaP-CR cells *in vitro*, but it significantly inhibited the growth of LNCaP-CR xenograft *in vivo*. Furthermore, 6G10A antibody also exerted strong anti-tumor activity against LNCaP-CR cells implanted orthotopically in murine prostate. Moreover, 6G10A antibody significantly inhibited the growth of various cancer xenograft models expressing CXADR *in vivo* such as human prostate DU-145, pancreatic BxPC3, and colorectal DLD-1 cancer cells. The study of its action demonstrated that 6G10A antibody exerted its anti-tumor activity through both ADCC and CDC activities. Thus, these results show that anti-CXADR 6G10A antibody is a promising candidate for cancer chemotherapy.

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Reversing the epithelial to mesenchymal transition with N-myc downstream regulated gene-1 and novel iron chelators in pancreatic cancer

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Pancreatic cancer is a devastating disease, as it remains one of the most challenging neoplasms to both diagnose and treat. Unfortunately, due to frequent late diagnosis, metastasis is likely to have occurred. By this stage, the treatment options for pancreatic cancer patients are limited and carry a poor success rate. Hence, new and more efficient therapies targeting metastasis are urgently needed. To this end, understanding the metastatic process and the molecules involved is crucial in order to develop more effective therapies for pancreatic cancer. Therefore, the focus of the current study was to examine the molecular mechanisms behind the epithelial to mesenchymal transition (EMT), a process known to facilitate metastasis in pancreatic cancer. The current study aimed to examine the capacity of the metastasis suppressor gene, *N-myc downstream regulated gene 1* (*NDRG1*), to promote an epithelial phenotype by attenuating oncogenic signalling pathways (*e.g.,* transforming growth factor beta (TGF-β) and nuclear factor kappa B (NF-κB)) known to facilitate the EMT.
Here, we report that NDRG1 is able to attenuate canonical NF-κB signalling in PANC1 pancreatic cancer cells through

i. inhibiting the IκB kinase (Iκκ) complex and its activation;
ii. preventing the phosphorylation and subsequent degradation of the inhibitory IκBα protein bound to the NF-κB complex, that would otherwise prevent its nuclear translocation and transcriptional activity; and
iii. NDRG1 reduces levels and nuclear expression of down-stream molecules of this latter pathway, namely claudin-1 and ZEB1.

In metastatic cancer cells, the tight junctional protein claudin-1 shifts from the plasma membrane and is mis-localised in the nucleus where it may facilitate the transcription of the E-cadherin gene repressor, ZEB1. Here we demonstrate, that NDRG1

i. reduces claudin-1 transcription and nuclear localisation, while possibly restoring claudin-1 membrane expression, and
ii. promotes an epithelial phenotype *via* up-regulating E-cadherin and down-regulating vimentin.

Importantly, NDRG1 can be up-regulated using a novel class of anti-cancer agents, namely the iron chelators, Dp44mT and DpC. To this end, these agents were shown to reduce activation of the Iκκ complex, and thus, attenuate NF-κB signalling. Interestingly, these agents were also found to reduce ZEB1 levels, and can be expected to reduce the repression of E-cadherin and inhibit metastasis.
In conclusion, the current study demonstrates the potential molecular mechanisms by which NDRG1 promotes an epithelial phenotype, and thus, inhibits the EMT. Additionally, this study demonstrates the promising application of novel iron chelators as anti-metastatic agents through their molecular targeting of the NF-κB signalling pathway.

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Vemurafenib alters glucose utilization in BRAF-driven human melanoma cells

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Background: Several BRAF/MEK inhibitors have recently been approved for the treatment of BRAF-driven melanoma and many more are undergoing clinical testing.
Inhibitors of BRAF and MEK attenuate the glycolic activity of BRAF mutant human melanoma cells but the downstream metabolic consequences of this action are not clear. Here we explore the alterations in glycolytic pathway fluxes induced by the BRAF inhibitor vemurafenib in BRAF mutant human melanoma cells using ^{13}C-labelled glucose and NMR spectroscopy.
Materials and Methods: BRAF^{V600D} WM266.4 human melanoma cells were treated with 0.2, 0.5 and 1μM vemurafenib (i.e. 1×, 2× and 5×GI_{50}, respectively) for 24 h in standard culture media and levels of extracellular lactate were determined by ^{1}H NMR spectroscopy. For pathway flux analysis, cells were treated with 2.5×GI_{50} vemurafenib in media containing 50% (12.5mM) [1-^{13}C] glucose. Following 24 h incubation, cell extracts were prepared and analysed by ^{13}C NMR spectroscopy to assess the levels of aqueous ^{13}C-labelled intermediates formed. ^{1}H and ^{13}C NMR data were acquired at 500MHz and metabolite levels normalized to cell number and an internal standard. Inhibitor action was verified by western blotting for P-MEK1/2 and P-ERK1/2 protein levels. Data represent mean±SD.