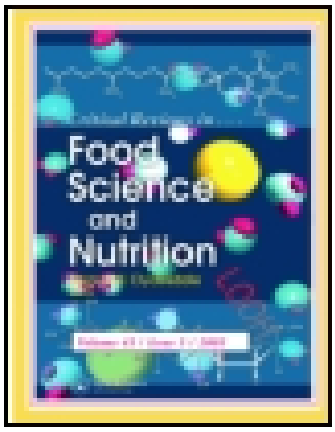

Critical Reviews in Food Science and Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bfsn20

Consumption of Red/processed Meat and Colorectal Carcinoma: Possible Mechanisms underlying the Significant Association

Ulf Hammerling[a], Jonas Bergman Laurila[b], Roland Grafström[cd] & Nils-Gunnar Ilbäck[e]

[a] Cancer Pharmacology & Computational Medicine, Dept. of Medical Sciences, Uppsala University and Uppsala Academic Hospital, SE-75185 Uppsala, Sweden

[b] Sahlgrenska Biobank, Gothia Forum, Sahlgrenska University Hospital, SE-413 45 Gothenburg, Sweden

[c] Institute of Environmental Medicine, the Karolinska Institute, PO Box 210, SE-171 77 Stockholm, Sweden

[d] Knowledge Intensive Products and Services, VTT Technical Research Centre of Finland, PO Box 106, FI-205 21 Turku, Finland

[e] Clinical Microbiology & Infectious Medicine, Dept. of Medical Sciences, Uppsala University and Uppsala Academic Hospital, SE-75185 Uppsala, Sweden

Accepted author version posted online: 07 Apr 2015.

To cite this article: Ulf Hammerling, Jonas Bergman Laurila, Roland Grafström & Nils-Gunnar Ilbäck (2015): Consumption of Red/processed Meat and Colorectal Carcinoma: Possible Mechanisms underlying the Significant Association, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2014.972498

To link to this article: http://dx.doi.org/10.1080/10408398.2014.972498

CONSUMPTION OF RED/PROCESSED MEAT AND COLORECTAL CARCINOMA: POSSIBLE MECHANISMS UNDERLYING THE SIGNIFICANT ASSOCIATION

Ulf Hammerling[1*], Jonas Bergman Laurila[2], Roland Grafström[3,4] and Nils-Gunnar Ilbäck[5]

[1] Cancer Pharmacology & Computational Medicine, Dept. of Medical Sciences, Uppsala University and Uppsala Academic Hospital, SE-75185 Uppsala, Sweden

[2] Sahlgrenska Biobank, Gothia Forum, Sahlgrenska University Hospital, SE-413 45 Gothenburg, Sweden

[3] Institute of Environmental Medicine, the Karolinska Institute, PO Box 210, SE-171 77 Stockholm, Sweden

[4] Knowledge Intensive Products and Services, VTT Technical Research Centre of Finland, PO Box 106, FI-205 21 Turku, Finland

[5] Clinical Microbiology & Infectious Medicine, Dept. of Medical Sciences, Uppsala University and Uppsala Academic Hospital, SE-75185 Uppsala, Sweden

* Corresponding author

Keywords: Dietary patterns, intestinal carcinogenesis, morphogenetic pathways, haem, nitrosyl-haem, N-nitroso compounds, fat peroxidation.

ABSTRACT

Epidemiology and experimental studies provide an overwhelming support of the notion that diets high in red or processed meat accompany an elevated risk of developing pre-neoplastic colorectal adenoma and frank colorectal carcinoma (CRC). The underlying mechanisms are disputed; thus several hypotheses have been proposed. A large body of reports converges, however, on haem and nitrosyl haem as major contributors to the CRC development, presumably acting through various mechanisms. Apart from a potentially higher intestinal mutagenic load among consumers on a diet rich in red/processed meat, other mechanisms involving subtle interference with colorectal stem/progenitor cell survival or maturation are likewise at play. From an overarching perspective, suggested candidate mechanisms for red/processed meat-induced CRC appear as three partly overlapping tenets: i) increased N-nitrosation/oxidative load leading to DNA adducts and lipid peroxidation in the intestinal epithelium, ii) proliferative stimulation of the epithelium through haem or food-derived metabolites that either act directly or subsequent to conversion and iii) higher inflammatory response, which may trigger a wide cascade of pro-malignant processes. In this review we summarize and discuss major findings of the area in the context of potentially pertinent mechanisms underlying the above-mentioned association between consumption of red/processed meat and increased risk for CRC.

INTRODUCTION

Globally, colorectal cancer (CRC) is the third most common malignancy among men and the second in women. Five-year disease survival can attain about 60 % in some developed countries, but much bleaker figures are seen in developing areas (Jemal *et al.*, 2011). This malignancy encompasses a range of slightly distinct pathological phenotypes, broadly segregated in hereditary and sporadic variants, but yet with many characteristics in common (Al-Sohaily *et al.*, 2012). Although certain variants of CRC are tightly connected with hereditary disposition, lifestyle factors have been identified as a profound implication in the development of this disease, as demonstrated by regional differences and migration studies (Center *et al.*, 2009). Notably, there is compelling evidence that consumers on a high red and/or processed meat diet are at moderately but significantly elevated risk of contracting intestinal malignancy, relative to those with a low intake of such foods (Aune *et al.*, 2013; Chan *et al.*, 2011). In a report from 2007, the World Cancer Research Fund/American Institute for Cancer Research Fund (WCRF) concluded that red and processed meat represent convincing risk factors for colorectal malignancy and the panel accordingly prescribes changes in dietary habits, with a particular emphasis on processed meat (WCRF/AICR, 2007). In fact, this resulted in the recommendation to limit intake of red meat, to not more than 500g/week, and to avoid consumption of processed meat. Actually, there is even support to the notion that the above-mentioned dietary pattern negatively influences prognosis for patients with diagnosed CRC (McCullough *et al.*, 2013). Moreover, the risks attached to this dietary pattern reportedly extend to cancers in several other organs as well, e.g. oesophagus, liver and lung (Cross *et al.*, 2007).

Conclusions made by the WCRF on processed meat and CRC are largely based on studies, which typically specify this food category in sweeping terms, thus entailing a weakness with respect to the specific curing or preserving processes that underlie the higher risk. There is a huge variety of processed meat described in the reports assessed and it is thus difficult to sort them by categories. Examples of parameters involved in the making of processed meat are curing (adding salt, nitrite and other additives), drying, smoking, cooking, and packing. Processed meat includes for instance bacon, ham (raw, smoked, or cooked), heated sausages like hot dogs, raw sausages (such as salami), bologna, blood sausages, liver paté (or liverwurst) and other patés and spread meat, luncheon meat and other cold cuts, canned meat and corned meat. All these different processes may generate products with various potential health hazards. Consequently, epidemiology studies on the relation between intake of processed meat and CRC are many times hard to evaluate and compare because of the huge variety within this broad food category and incomplete description of the products specifically investigated. No systematic epidemiology study has compared different kinds of processed meat in relation to CRC incidence.

Despite an unequivocally demonstrated association between the aforementioned consumption pattern and an elevated risk of contracting CRC through numerous epidemiology studies there is as yet no scientific consensus established on the specific underlying mechanisms/factor(s) (Corpet, 2012). Even though few experimental investigations have laid a sole focus on processed meat, the accumulated number of studies undertaken on red meat has nonetheless rendered it possible to propose some potential mechanisms. Consequently, based on available and experimental data there is no known single mechanism that can explain how red and processed meat act in the development of CRC. The potential mechanisms for red/processed meat-induced

CRC have, though, been elaborated on, which broadly fall in three - in part overlapping - main categories: those based on an enhanced mutagenic intestinal environment, those favoring certain growth-promoting dietary components ï centered on either haem or arginine - and those focused on an induced intestinal inflammatory response (Azadbakht and Esmaillzadeh, 2009; Bingham *et al.*, 2002; Zell *et al.*, 2012). Animal fat itself and lipid oxidation products are two additional issues elaborated on in the literature, in this context. Nonetheless, a large body of reports supports the notion that haem and particularly nitrosyl haem, the latter derivative formed as a consequence of meat curation with sodium nitrite, are major contributors to food-dependent intestine carcinogenicity. These factors may act either alone or ï more commonly suggested ï through induced N-nitrosation compounds (NOCs) or oxidated lipids (Corpet, 2011; Cross *et al.*, 2003; Pierre *et al.*, 2004; Sesink *et al.*, 1999). Further, human intestinal transcriptomic response to red meat consumption discloses a wide array of gene expression changes, including those of proliferative stimulation, apoptosis and tissue morphogenesis (Hebels *et al.*, 2012; Ijssennagger *et al.*, 2012). Prospects to arrive at deeper understanding of the underlying mechanisms are promising given the new and powerful tools to pursue molecular and mechanistic events at a systems biology level (Edberg *et al.*, 2012; Hammerling *et al.*, 2009; Sturla *et al.*, 2014; Thomson *et al.*, 2013). Moreover, recently reported molecular characterization of changed genes and deranged pathways typical of CRC should be highly conducive to continued pursuit of the pertinent mechanisms, in the context of red/processed meat-associated risk (Armaghany *et al.*, 2012; Ashktorab *et al.*, 2010; Muzny *et al.*, 2012).

The cancer stem cell (CSC) model ï also known as the hierarchy concept - has endowed tumor biology with a major conceptual extension over the last decade. The core paradigm,

analogous with that of normal stem cells, involves postulation of a cellular hierarchical organization in malignant lesions, in which only a small cellular subset carries the potential to initiate tumorigenesis and sustain all facets of malignancy (Puglisi *et al.*, 2013; Visvader and Lindeman, 2012). The human intestine epithelium is characterized by rapid cellular turnover and is thus in constant need of replacement from progenitors, a process highly dependent on the accurate functioning of key morphogenetic proteins. The Wnt/ϐ-catenin signal transduction cascade in particular, but likewise those of Hedgehog, Notch and bone morphogenetic proteins (BMPs) are major morphogenetic pathways implicated in normal and malignant intestinal development (Miyamoto and Rosenberg, 2011; Varnat *et al.*, 2009; White *et al.*, 2012). It seems likely that an impact on such signaling cascades, through several routes, is implicated in the complex mechanisms behind red/processed meat-related risk of CRC development. This assumption is in part rooted in the requirement of fine-tuned regulation of stem cell proliferation and maturation, supported by a stem cell niche, but with several inroads to attack by exogenous bioactive substances (Medema and Vermeulen, 2011; Visvader, 2011).

This review aims at summarizing findings in experimental and clinical nutrition studies on the above-mentioned dietary patterns as well as ï with a much stronger emphasis - surveying major avenues to potential mechanisms behind elevated risk for CRC development associated with a high consumption of red and processed meat. A brief overview of CRC pathology, including various molecular components and processes pertinent to intestinal carcinogenicity and sustenance of a malignant phenotype, is included to assist digestion of the following outline and discussion on several, in many cases interlaced, molecular signaling cascades of potential relevance to the diet-induced colorectal malignancy.

BRIEF OUTLINE OF HUMAN COLORECTAL CARCINOMA

Some facets of intestinal physiology and pathology

The mammalian intestine is composed of several histologically distinct layers, such as mucosa, sub-mucosa muscle layer and serosa. The apical intestinal surface comprises a large number of invaginations, referred to as crypts of Lieberkühn. Basal parts of those crypts harbor pluripotent stem cells, which gradually acquire more mature cellular functions along their migration towards the villus site. These stem cells possess the capacity to mature to anyone among four major differentiated epithelial cell types: enterocytes (with an absorptive function), goblet cells (secreting mucus), enteroendocrine cells (secreting peptide hormones) and Paneth cells (producing anti-microbial substances) (Binder, 2009; Shaker and Rubin, 2010). This composite organo-renewal process is dependent on a fine-tuned orchestration of various extracellular stimuli and signal transduction cascades, of which the latter include Wnt, Hedgehog, Notch and bone morphogenesis protein (BMP) pathways (van der Flier and Clevers, 2009).

Colorectal carcinoma (CRC), a set of malignant disorders arising from epithelial cells lining the colon or rectum of the gastrointestinal tract, is the third leading cause of cancer globally and the second malignancy-attributed mortality rate in Europe (Ferlay *et al.*, 2013; Soerjomataram *et al.*, 2012). Although survival rates for early stage CRC are reasonably good, dismal prognosis follows tumor engagement of lymph nodes and nearly half of the patient population with CRC develop disease relapse. As typical of cancers in general CRC is a heterogeneous disease, wherein both hereditary and environmental or life-style factors interplay. Notably, adiposity and

sedentary lifestyle as well as adherence to a Western dietary pattern are known to predispose for CRC (Fung *et al.*, 2003; Renehan *et al.*, 2008). Progress from health to disease involves the sequential development through several stages from pre-neoplastic lesions, commonly appearing as colorectal adenoma, to overt malignancy (Fearon, 2011; Fearon and Vogelstein, 1990; Leslie *et al.*, 2002) [Figure 1]. Adenocarcinoma is the most prevalent colon cancer cell type, but the traditional adenoma to carcinoma sequence may account for only about 60 % of all cases. In recent years the serrated neoplastic pathway to CRC, i.e. tumors developed from precursor lesions referred to as serrated adenomas/polyps and encompassing up to 35 % of carcinomas, has gained increased recognition (Fleming *et al.*, 2012; Snover, 2011). Four major pathways have been proposed as implicated in the development of CRC ï genomic instability, genomic mutations, microRNA and epigenetic changes (Kanthan *et al.*, 2012). A large set of genetic alterations have been identified in resection polyp/cancerous intestinal tissue and in human colorectal cell lines. Recently, a comprehensive set of mutated or otherwise deranged genes, associated with either colorectal adenoma or manifest CRC, was reported and many functionally pivotal relationships between identified genetic aberrations and malignancy are known (Al-Sohaily *et al.*, 2012; Muzny *et al.*, 2012).

In the clinical setting the Dukes A through D disease stage system is still in practice, but a more recently developed scheme - AJCC TNM - has lately gained increased clinical popularity (Puppa *et al.*, 2010). Broadly, CRC may be segmented in two main classes - hereditary and sporadic forms ï each encapsulating various subtypes. Major varieties of the former category are Familial Adenomatous Polyposis and the much more common Lynch syndrome, also known as Hereditary NonPolyposis Colorectal Cancer. Peutz-Jeghers Syndrome and Juvenile Polyposis

Syndrome are other hereditary CRC varieties (Fearon, 2011; Rustgi, 2007). The sporadic cases (which are much predominant), however, may present with many features of inherited varieties. For instance, dysregulation of the Wnt/ß-catenin signalling cascade is a common feature shared by these two classes (Fodde *et al.*, 2001). CRC can also be stratified according to subsets of major genomic instability pathways: the microsatellite instability (MSI) and the CpG Island Methylator Phenotype (CIMP) path. Based on specific combinations of MSI (MSI^+, MSI2) and CIMP ($CIMP^+$, CIMP2), four major molecular CRC subtypes have been proposed (Kang, 2011). More recently, CRC classification systems were inferred from gene expression data, revealing either three or five interrelated major subtypes, largely depending on the classifiers and clustering algorithms used to process data. The latter scheme (five classes) arrived at ñStem-likeò, ñTransit Amplifyingò, ñGoblet-likeò, ñEnterycyteò and ñInflammatoryò groups, with good and poor disease-free survival data for ñGoblet-likeò and ñStem-likeò CRCs, respectively (Sadanandam *et al.*, 2014).

CERTAIN KEY MOLECULAR EVENTS AND INTERACTIONS RELATED TO MOLECULAR CRC PATHOGENESIS

Various genes and pathways implicated in CRC

Up to 80 % of all sporadic colorectal adenomas and carcinomas as well as more than 90 % of Familial Adenomatous Polyposis variants, feature somatic mutations or otherwise inactivating changes of either *APC* (*adenomatous polyposis coli*) or *CTNNB1* - key genes of the Wnt signaling cascade (Segditsas and Tomlinson, 2006). Mitogenic signaling through the EGF (epidermal growth factor) receptor (EGFR) pathway, commonly bypassing the receptor itself, is

perceived as crucial to CRC maintenance and progression, but the receptor itself is rarely genetically activated in CRC (Wood *et al.*, 2007). Key proteins of this signaling cascade are Kras/Braf, on the one hand, and PI3K/Akt (phosphatidylinositol-4,5-bisphosphate 3-kinase/Protein Kinase B), on the other, each pair being part of a reasonably distinct pathway, albeit with several inter-route connections (De Roock *et al.*, 2011; Markowitz and Bertagnolli, 2009). *KRAS* and *BRAF* themselves are commonly subject to activating mutations in CRC tissue, the former gene being perceived as contributing to adenoma promotion, rather than initiation (Wood *et al.*, 2007). Up to 25 % of human CRCs display somatic activation mutations in *PIK3CA* (*phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha*; part of the PI3K/Akt pathway), thereby increasing intracellular levels of proliferation- and survival-promoting phosphatidylinositol -3,4,5-triphosphate (Armaghany *et al.*, 2012; Samuels *et al.*, 2004). Further, the tumor suppressor genes *TP53* (*tumor protein p53*) and *DCC* (*deleted in colorectal carcinoma*) are frequently inactivated through somatic mutations, resulting in weakened apoptotic machinery (Kanthan *et al.*, 2012; Mao *et al.*, 2012). Many additional genetic derangements are likewise frequently seen in intestinal malignant lesions, such as those of the *TGFBR2* - encoding TGF-ß (transforming growth factor ß) receptor type 2 as well as the tumor suppressor *PTEN* (*phosphatase and tensin homolog*) (Armaghany *et al.*, 2012; Muzny *et al.*, 2012).

Epigenetic alterations are frequent early events in colorectal carcinogenesis. Certain DNA repair genes ï especially *hMLH1* (*DNA mismatch repair protein*) and *MGMT (O^6-methylguanine DNA methyltransferase*) and some major tumor suppressor genes, including *APC*, are commonly inactivated in CRC through aberrant promoter hypermethylation (Kanwal and Gupta, 2012;

Tanaka *et al.*, 2007). Abnormal expression of several microRNAs (miRNAs), causing disruption of the expression of many protein-encoding genes, is likewise seen in many CRCs (Balaguer *et al.*, 2010; Deng *et al.*, 2011).

Wnt signalling and CRC

The Wnt pathway, which can follow either a canonical route, i.e. dependent on ϐ-catenin, or any among several non-canonical sequences of activation, is fundamental to accurate maturation of physiological stem cell populations in various tissues (Wend *et al.*, 2010). It is likewise implicated in epithelial malignancies - typified by CRC - and the associated cancer stem cells (Fearon, 2011; Polakis, 2012; White *et al.*, 2012). The canonical route is briefly as follows: In the absence of Fzd/LRP (frizzled/low density lipoprotein receptor-related protein) heterodimer receptor stimulation with ligand, ϐ-catenin occurs as an inactive member of a hexameric cytoplasmic aggregation composed of ϐ-catenin, APC, GSK-3ϐ (glycogen synthase kinase 3 beta), Axin and two more proteins. This is a normal state of differentiated healthy tissue and involves incessant tagging of ϐ-catenin for destruction. Receptor engagement halts this steady-state and thus triggers the accumulation of ϐ-catenin in the cytoplasm, where it complexes with the LEF (lymphoid enhancer family) and TCF (T-cell factor) family of transcription factors and subsequently appears in the nucleus. At this location the new ϐ-catenin complex induces the expression of various Wnt-regulated genes, including *CMYC* and *cyclin D1* (Scholer-Dahirel *et al.*, 2011; Wend *et al.*, 2010). The activated Wnt cascade is likewise linked to induction of *ODC* (*ornithine decarboxylase-1*) and thus stimulation of the cognate polyamine metabolism (Fultz and Gerner, 2002; Martinez *et al.*, 2003). In diseased intestinal tissue, for instance expressing either of mutant *APC*, *GSK-3β*, *Axin* or even *CTNNB1* itself (ß-catenin gene), however, the ϐ-

catenin inactivation mechanism is drastically attenuated or even abolished, thereby promoting aberrant transcriptional activation of the aforementioned and additional genes of importance to growth control and differentiation (Kanthan *et al.*, 2012; White *et al.*, 2012). A large majority of sporadic and hereditary CRCs involve either dys-functional APC or ß-catenin, thereby (partially) activating the normal Wnt cascade constitutively (Al-Sohaily *et al.*, 2012; Armaghany *et al.*, 2012). Wnt signaling is also regulated by a diverse set of soluble inhibitors of Wnt ligands, such as that encoded by *Wif1* (*Wnt inhibitor factor-1*). The Wnt cascade of normal and malignant cells holds, however, much higher complexity than outlined above. An excellent in-depth overview of the area appears in a recent review by Holland and colleagues (Holland *et al.*, 2013).

Involvement of Hedgehog, Notch and BMP pathways in CRC

Similar to the Wnt pathway, the Hedgehog (Hh) cascade can follow either of a canonical or anyone among several non-canonical pathways. The former trajectory involves activation triggered by anyone of three Hh ligands (Sonic, Desert or Indian), which thus releases an inhibitory component - the tumour suppressor Patched1 (Ptch1) - from Smoothened (Smo), which ï like Ptch1 ï is a transmembrane G-protein-coupled receptor. Subsequent to release of another blockage of this pathway, the Suppressor of fused (SUFU), Smo allows the Glioma-associated oncogene (Gli) to trigger transcriptional activation of Gli-activated genes (Marini *et al.*, 2011). At least one of the alternative routes allow for activation by Smo and Ptch1 directly, i.e. without Hh ligand engagement (Riobo, 2012). Dys-regulation of the Hedgehog pathway is highly associated with a relatively restricted set of human malignancies, notably medulloblastoma, basal cell carcinoma and glioblastoma, but has likewise been demonstrated as implicated in CRC (Arimura *et al.*, 2009; Sun *et al.*, 2013; Varnat *et al.*, 2009). The Notch

system, likewise critical to normal colorectal epithelial maturation, plays a key role in regulating cell-cell communication (Miyamoto and Rosenberg, 2011). Notch receptors, occurring as four highly sequence-similar single-pass trans-membrane proteins (Notch 1 through 4), are activated through interaction with ligands from the delta and jagged family of proteins. Receptor engagement with ligand triggers a series of proteolytic cleavage steps of Notch, of which that accomplished by ɔ-secretase is the most widely reported reaction as well as subject of experimental drug intervention (Takebe *et al.*, 2011). An accordingly truncated protein fragment (NICD) thus becomes released from the plasma membrane to subsequently appear in the nucleus, where it binds to either of several sets of transcription factors. These complexes ultimately activate an array of genes, the *Hairy/Enhancer of Split* (*HES*) family, *cyclin D1* and *CMYC* being some outstanding representatives (Iso *et al.*, 2003; Ronchini and Capobianco, 2001; Weng *et al.*, 2006). Actually, Notch and Wnt/ɓ-catenin cascades cooperate in malignancy so as *APC* mutant intestinal adenoma stem cells would rather differentiate to goblet cells, unless supported by Notch signaling (Fre *et al.*, 2009; van Es *et al.*, 2005). In some contrast to the Wnt/ɓ-catein pathway that of Notch seems largely be implicated in tumor initiation, but not progression, as revealed by experimental findings in a mouse model and in cell lines (Fre *et al.*, 2009; Sikandar *et al.*, 2010).

The BMPs (bone morphogenetic proteins), lastly, are a heterogeneous family of multifunctional factors of the TGF-ɓ superfamily of morphogenetic proteins. The BMPs act through binding to a heterodimeric transmembrane receptor complex. Upon receptor activation, the associated SMAD (small mothers against decapentaplegic) proteins become phosphorylated and subsequently complexed with SMAD4, which carries them to the nucleus where they

regulate gene transcription. In the healthy intestine, expression of BMPs is high in the colon crypt tops where differentiated cells are most abundant, whereas several BMP inhibitors are expressed at the crypt base, a location of higher proliferative and less mature cellular states (Ashley, 2013; Hardwick *et al.*, 2008). Mutations in either *BMPR1A* (*BMP receptor 1A*) or *SMAD4* has been associated with Juvenile Polyposis, which leads to the formation of hamartoma lesions, a cancerous precursor lesion. More recent findings, however, support the notion that mutations in this pathway are likewise common among sporadic colorectal cancers (Hardwick *et al.*, 2008).

Epigenetic mechanisms and CRC

Epigenetics is referred to as the research field of heritable but potentially reversible changes in gene expression, attributable to specific covalent modifications of chromatin structure and DNA, i.e. changes that do not involve alterations in the primary DNA sequence (Skinner *et al.*, 2010). Major epigenetic mechanisms include DNA methylation and changes to the nucleosome through histone modification, but the regulation of gene expression by miRNAs is likewise part of this intricate area (Portela and Esteller, 2010; Suzuki *et al.*, 2013). Methylation of DNA typically involves the addition of methyl groups at cytosine residues positioned next to a guanine, so called CpG dinucleotides. Some of these motifs are clustered in regions, referred to as ñCpG islandsò, which appear in gene promoters and surround transcription start sites. In normal cells, the CpG islands of such promoter regions are scarcely modified ï less than 10 % show this alteration. Moreover, an appreciable fraction (about 40 %) of human gene promoters is devoid of CpG motifs, but yet being amenable to epigenetic regulation through DNA methylation. The vast and scattered chunks of repetitive DNA sequences across the human

genome are, however, extensively methylated, which is believed to confer genomic stability (Portela and Esteller, 2010; Tsai and Baylin, 2011) . Modification of histone N-terminal tails is an even more intricate layer of epigenetic mechanisms, involving an array of covalent changes, such as acetylation, methylation, phosphorylation and ADP ribosylation, the first two mechanisms being most intensely investigated. Generally, acetylation of histone tails renders the chromatine structure more accessible to transcription, whereas the effect of lysine modification depends on the residue methylated (Sharma *et al.*, 2010). Notably, the methylation state of DNA is dependent of the specific modifications of certain histone proteins (Cheng and Blumenthal, 2010).

Well-tuned epigenetic machinery is crucial for a variety of physiological processes, such as foetal development as well as tissue and cell differentiation. However, aberrant epigenetic modifications are integral to the pathology of several disorders, including those of the CRC category, (Peltomaki, 2012; Sharma *et al.*, 2010). Seemingly, such changes are an early event in the development of CRC and other malignancies, but likewise implicated in tumor progression. Early research within this area revealed global hypomethylation in colon adenomas and adenocarcinomas (Feinberg *et al.*, 1988). Subsequent investigation has disclosed a rich assortment of regional hypermethylation modifications in CRC. For instance, the ñCpG island methylator phenotypeò (CIMP) relates to frequent hypermethylation across multiple CpG sites and is associated with specific pathological characteristics, including microsatellite instability, of certain colorectal cancers (Tanaka *et al.*, 2007; Toyota *et al.*, 1999; Weisenberger *et al.*, 2006). Important DNA repair genes, especially *hMLH1* and *MGMT*, are commonly inactivated in human CRC, through CpG hypermethylated promoters, leading to genome instability (Kane *et*

al., 1997; Tanaka *et al.*, 2007). Moreover, promoter hypermethylation is a major inactivation route for tumor suppressor genes in malignant diseases, thereby leading to (or contributing to) tumor initiation or progression (Ushijima, 2005). In CRC, several such genes, such as *APC*, *Rb*, $p16^{INK4A}$, often carry aberrant promoter methylation (Esteller *et al.*, 2000; Kanwal and Gupta, 2012). Furthermore, certain miRNAs are frequently down-regulated through promoter methylation in pre-neoplastic and cancerous intestinal tissue, in some cases leading to microsatellite instability (Deng *et al.*, 2011). According to a recent report, about 80 % of all colon carcinomas show miR-137 silencing, due to CpG island methylation of its encoding DNA (Balaguer *et al.*, 2010). Conversely, certain miRNAs, as typified by miR-21, are commonly up-regulated in malignant cells. In CRC tissue, this oncomir is reportedly targeting ï and thereby attenuating ï several tumor suppressor genes, such as *PTEN* and *PDCD4* (*programmed cell death protein 4*) (Asangani *et al.*, 2008; Zhu *et al.*, 2008). Several dys-regulated miRNAs have been attributed to various histological types of CRC (Deng *et al.*, 2011).

DIETARY HABITS AND CRC

Clearly, hereditary causes of CRC, although involving diverse mutations and with variable penetrance, relate to - in most cases - well-known molecular changes in the hostôs genome setup (Al-Sohaily *et al.*, 2012; Armaghany *et al.*, 2012; Kanthan *et al.*, 2012). The etiology of sporadic CRC variants, though, is more difficult to disentangle and thus needs dedicated epidemiology investigation in tandem with molecular-mechanistic studies. Over the last two decades, an appreciable number of nutrition epidemiology studies on this issue have been reported and already those conducted in early times indicate a likely connection between Western-type eating

habits, particularly red/processed meat consumption, and an elevated risk of contracting CRC (Fung *et al.*, 2003; Kesse *et al.*, 2006; Meyerhardt *et al.*, 2007; Norat *et al.*, 2002; Slattery *et al.*, 1998). Conversely, foods rich in fruit and vegetables, i.e. low-fat and high-fiber patterns, are found to protect against colorectal cancer (van Duijnhoven *et al.*, 2009; Wirfalt *et al.*, 2009). Dietary patterns high in poultry and (occasionally) fish have likewise been observed as slightly inversely correlated with such disease risk (Chao *et al.*, 2005; Daniel *et al.*, 2011; English *et al.*, 2004; Miller *et al.*, 2013; Norat *et al.*, 2005). These results have extended the knowledge and demonstrated a tendency towards an independent effect of poultry and fish, apart from a more clear-cut inverse relationship to meat/poultry-fish replacement.

The WCRF/AICR panel concluded already in 2007 that red and processed meat are risk factors for developing CRC and thus prescribed lower intake of such foods: "Limit intake of red meat and avoid processed meat" (WCRF/AICR, 2007). According to the WCRF evaluation, largely based on 17 cohort studies, an increase in relative risk was shown in some studies with servings of 700 g/week of red meat and servings of 350 g/week of processed meat. A dose-response relationship was likewise apparent (WCRF/AICR, 2007). In many countries the consumption of red and processed meat is considerably higher than the aforementioned intake levels and thus exceeds those recommended by the WCRF. Hazard risk ratios vary across reports, but appear to centre around 1.20 for fresh red meat and about 1.30 for processed meat in recently conducted large meta-analyses (Aune *et al.*, 2013; Chan *et al.*, 2011). Some earlier research reports, however, arrive at higher hazard ratios - about 1.4-1.5 and 1.5-1.7 for fresh red and processed meat, respectively - for highest *versus* lowest intake (English *et al.*, 2004; Norat *et al.*, 2005). Actually, Santarelli and colleagues have, based on three meta-analyses among a

selection of epidemiology studies, concluded that the risk associated with consumption of one gram of processed meat is two to ten times higher than the risk associated with one gram of fresh red meat (Larsson and Wolk, 2006; Norat *et al.*, 2002; Sandhu *et al.*, 2001; Santarelli *et al.*, 2008). Furthermore, a near-linear risk increase up to about 140 g consumed meat/day was inferred from a large set of epidemiology studies (Chan *et al.*, 2011). In some disparity with the former report a subsequent investigation arrived at a non-linear dose-response relationship, attaining an asymptotic level at about 80 g/day (Aune *et al.*, 2013). Moreover, test volunteers given processed meat showed 2-3 times higher NOC levels than those on a fresh meat diet (Joosen *et al.*, 2009).

Furthermore, several malignancies, besides those of the intestinal tract, can be correlated to the consumption of red/processed meat. A significant risk elevation of 20 % to 60 % was reported to follow from red and processed meat consumption, not solely with respect to CRC incidence but also extending to several additional malignancies, such as those of esophagus, liver and lung (Cross *et al.*, 2007). A subsequent report, based on a large prospective study, showed up to 20 % reduced risk of cancers in various organs, apart from the intestine, following estimated substitutions of red with white meat (Daniel *et al.*, 2011). The dietary habit has also been observed to associate with higher incidence of colorectal adenoma, a precursor state of CRC (Sinha *et al.*, 2005). Moreover, CRC patients with high intake of processed and read meat, prior and subsequent to clinical diagnosis, were at higher risk of CRC-associated mortality (McCullough *et al.*, 2013).

To summarize, there is a large body of nutritional epidemiology evidence in support of dietary patterns rich in red and processed meat being associated with a moderate but nonetheless

significantly elevated risk of contracting CRC; processed meat displaying the tightest statistical connection (Aune *et al.*, 2013; Santarelli *et al.*, 2008; WCRF/AICR, 2007) .

PURSUING MECHANISTIC PROCESSES IMPLICATED IN CRC TUMOUR INITIATION AND PROGRESSION IN THE CONTEXT OF RED/PROCESSED MEAT CONSUMPTION

Both genotoxic/mutation and developmental signal transduction views are needed to approach the potential mechanisms

Multistep development of human tumors involve the induction of genetic and epigenetic changes to DNA leading to aberrant gene expression, either through malfunction of the affected genes themselves or through accordingly induced abnormal regulation of other genes of importance to key cellular function(s) (Hanahan and Weinberg, 2011). Red and processed meat are carriers of several such genotoxic and mutagenic substances, of which N-nitroso compounds and certain oxidized lipid species are perceived as imposing a substantial challenge to intestinal genomic integrity (Feng *et al.*, 2003; Gratz *et al.*, 2011). Typically, a gradually increased accumulation of such lesions in critical genes, jointly with cellular genetic instability, accompany an initial appearance of pre-neoplastic polyps, which - upon subsequent deleterious alterations of proto-oncogenes or suppressor genes - may develop into overt carcinoma (Kanthan *et al.*, 2012).

The intestine epithelium undergoes continuous regeneration and turnover at a high pace, involving multi-linear maturation of crypt stem cells. This mechanism is strictly dependent on the accurate development of stem cells into mature epithelial lineages. Moreover, there is strong

genetic support to the notion that stem cells are the cells-of-origin in intestinal cancers through the sequential acquisition of mutations in the genome of this cell type (Barker *et al.*, 2009; Visvader, 2011). Thus, is comes as no surprise that colorectal stem cells (CSCs) are highly implicated in human colorectal malignancy (Wilson *et al.*, 2011). Further, observations made in various experimental settings strongly support the notion that (sub-populations of) CSC and bulk tumor cell populations occur in mutual equilibrium, which can be changed in either direction by various endogenous or exogenous compounds (Iliopoulos *et al.*, 2011; Schwitalla *et al.*, 2013). Moreover, Vermeulen and colleagues have reported a myofibroblast-mediated acquisition of drastically enhanced CSC clonogenicity and tumorigenicity, paralleled by mounted Wnt expression; an effect which could be mimicked by myofibroblast-culture conditioned medium (Vermeulen *et al.*, 2010). Other findings corroborate the importance of microenvironmental factors in the regulation of stem cells, which thus can be modulated by external agents or biomolecules, thereby promoting tumor initiation and progression (Medema and Vermeulen, 2011).

As touched upon above, there is a major involvement of deregulated Wnt signal cascade in both hereditary and spontaneous CRC (Scholer-Dahirel *et al.*, 2011; White *et al.*, 2012). Other signal transduction cascades implicated in the regulation of stem cells - notably those known as Hedgehog and Notch - communicate with that of Wnt and are likewise reported as key pathways of colorectal carcinogenesis (Sikandar *et al.*, 2010; Varnat *et al.*, 2009). A study on human subjects suggests induced intestinal gene expression of the Notch and Wnt signaling cascades, in response to diets rich in red meat (Hebels *et al.*, 2012). Further, investigations on haem-fed mice revealed diet-mediated suppression of Wnt-inhibition as well as reduced expression of Ihh and

BMP2 factors (Ijssennagger *et al.*, 2012). These observations lend credence to an involvement of dys-regulated morphogenetic pathways of intestinal stem cells, in the dietary-CRC incidence context. Such changes may have arisen through direct or indirect impact on the regulation of the key morphogenetic proteins, as a consequence of the aforementioned risky dietary pattern, thus potentially mounting stem cell susceptibility to genetic alterations through intensified or prolonged exposure to a mutagenic environment. Thus, actions on stem cells and their malignant counterparts - cancer stem cells - apart from those of genetic insult, may deserve more attention in the continued quest for underlying factors and mechanisms of the risk pattern attached to red and processed meat.

FOOD RELATED CRC IN THE CONTEXT OF CHEMICALS-INDUCED GENETIC INSULT

Clusters of genotoxic and/or mutagenic substances in red and processed meat

Various molecular mechanisms behind the observed statistical association between red/processed meat intake and an increased risk of developing CRC have been proposed. Three chemical substance categories are generally perceived as being attributed to this risk: i) Heterocyclic amines (HCAs), which are formed upon heating mixtures of amino acids and sugars to high temperatures, either alone or jointly with polycyclic aromatic hydrocarbons (PAHs), typically produced upon smoking or barbequing animal-derived raw commodities, ii) N-nitroso compounds (NOCs), which can arise either as a consequence of cooking or through endogenous (intestinal) formation, i.e. through N-nitrosation of amines or amides derived from acid- and enzyme digested proteins as well as iii) nitrites/nitrates, commonly appearing on such foods

owing to preservation or curing purposes (Cross *et al.*, 2010; Sinha *et al.*, 2005; Tricker and Preussmann, 1991) [Figure 2]. Among the most nurtured hypotheses on food-mediated intestinal carcinogenesis are those built on HCAs and particularly nitroso amines (Bingham *et al.*, 2002; Sugimura *et al.*, 2004). The assumed connection to CRC is rooted in a mutagenic property of many among these compounds, as evidenced by results from a rather broad array of *in vitro* and *in vivo* assays. Applicability to the red/processed meat-CRC association is, however, compounded by several circumstances. Notably, certain mutagenic species of heterocyclic amines occur in cooked red meat, but are likewise found in poultry, a food not associated with elevated risk of intestinal malignancy (Bingham *et al.*, 2002; Miller *et al.*, 2013). Further, the ingested levels of HCA through food themselves is far from sufficient to explain human cancer, i.e. the levels of such heterocyclic amines needed to trigger genotoxic or mutagenic changes are several magnitudes higher than those found on cooked foods, but might nonetheless contribute jointly with other mutagens to create a cancerogenic intestine environment (Stavric, 1994; Sugimura *et al.*, 2004). Moreover, certain PAHs are potent carcinogens, but occur at low levels in regularly cooked meat foods. Barbequed meat, however, contain higher amounts, but cereals are nonetheless the major source of ingested PAHs (Phillips, 1999). Conversely, high levels of intestinal NOCs, largely formed via myoglobin-bound haem, are observed following ingestion of red or processed meat (contrary to white meat or fish), thereby promoting various oxidative reactions (Bingham *et al.*, 2002; Cross *et al.*, 2003; Hughes *et al.*, 2001). Some of those molecular changes appear as O^6-CMG adducts, which are not readily removed by endogenous repairing enzymes (Lewin *et al.*, 2006). Moreover, meat-induced NOCs have been shown to bring various additional changes to the intestinal environment, e.g. certain lipid peroxidation end

products, which are recognized as candidate carcinogens (Bastide *et al.*, 2011; Corpet, 2011; Cross *et al.*, 2003; Kuhnle *et al.*, 2007). One of those ï 4-hydroxynonenal (4-HNE) ï is known to interfere with several signaling transduction pathways and a bioconversion product (1, 4-hydroxynonenane mercapturic acid) appears in urine, in proportion to ingested haem (Pierre *et al.*, 2006) [Figure 2].

Red meat consumption and NOC-mediated cellular insult

A series of reports consistently show higher intestinal NOC levels following high meat diets, compared with those of poultry or fish (Bingham *et al.*, 2002; Cross *et al.*, 2003; Hughes *et al.*, 2001; Joosen *et al.*, 2009; Kuhnle *et al.*, 2007). Notably, output of these highly bioactive compounds, collectively referred to as apparent total N-nitroso compounds (ATNC), from volunteers consuming increasing levels of red meat displayed high correlation to the daily intake, from 60 g/day up to 240 g/d (Bingham *et al.*, 2002). An early investigation of human volunteers did, however, not find any association between genotoxicity assay outputs and levels of ATNC (Cross *et al.*, 2006). Subsequent reports, likewise based on findings in healthy volunteer consumers, observe higher intestine NOC levels in subjects on a red meat-rich diet, relative to those of either vegetarian or fish consumption patterns, but without augmented markers of fecal water genotoxicity (Joosen *et al.*, 2009; Joosen *et al.*, 2010). Gene expression and subsequent biochemical pathway analysis of a diverse set of NOCs, using an *in vitro* colorectal cell experimental system, revealed negligible effect of nitrosamides, relative to that of nitrosamines. Members of the latter NOC category, however, were found to activate a rich set of transcripts of the cell cycle and apoptosis pathways in Caco-2 cells and in biopsies of human volunteers (Hebels *et al.*, 2009; Hebels *et al.*, 2011). An ensuing report of the same research group showed

that a diet rich in red meat, in patients suffering either from inflammatory or irritable bowel syndrome, correlates positively to activation of a range of pro-neoplastic genes, as revealed by gene expression analysis. These mRNA species included those of the Wnt and Notch pathways, both essential to physiological regulation of development, but likewise implicated in colorectal carcinogenesis (Hebels *et al.*, 2012; Reedijk *et al.*, 2008). Contrary to most reports on this particular issue, however, no rise in ATNC (fecal water) was seen after the intervention (Hebels *et al.*, 2012). The absence of a relationship between dietary (red meat)-induced effects on gene expression and NOC in this report may either be rooted in the study subjectôs intestinal pathology, curtailing additional oxidative load, or simply reflect a mechanism seemingly not strictly dependent on NOC.

Haem can efficiently induce the formation of intestinal N-nitroso compounds

Owing to its much higher abundance in red meat, relative to poultry or any other white meat food, haem has been proposed as a major causative agent of the dietary-associated CRC, despite conflicting reports on the implication of NOCs in disease promotion and a catalytic role of haem in the formation of reactive oxygen species (Bingham *et al.*, 2002; Cross *et al.*, 2003). There is, however, a growing body of evidence supporting the notion that haem, and nitrosyl haem to an even higher extent, trigger the gastro-intestinal formation of a set of NOC compounds, including S-nitrosothiols, nitrosamines and nitrosamides [Figure 2]. These ATNCs catalyze the formation of various promutagenic and toxic adducts of which O^6-CMG and O^6-methylguanine (O^6-MG) seemingly are the most severe (Gottschalg *et al.*, 2007; Lewin *et al.*, 2006). Such adducts were identified in stool colonocytes of volunteers on a high red meat diet (Lewin *et al.*, 2006). Regardless of whether mutagenic action is a major mechanism, a connection between dietary

exposure to haem and CRC incidence lends further credence from numerous additional findings. For instance, haem induced more than twice the frequency of colonic mucin-depleted foci in treated rats, relative to that of control rats (Pierre *et al.*, 2010). Dose-dependent impact of meat intake among human volunteers on colonic N-nitrosation and a likewise dose-dependent action of haem on numbers of mucin depleted colonic foci in azoxymethan-treated rats have been reported (Hughes *et al.*, 2001; Pierre *et al.*, 2004). The latter study found the highest numbers of pre-neoplastic intestinal lesions in animals fed blood sausage, with a gradually declining order in subjects fed meat or poultry (Pierre *et al.*, 2004). Iron alone, however, is devoid of this association (Cross *et al.*, 2003). Further, several nutritional epidemiology studies have found a slight inverse statistical correlation between chicken consumption and CRC incidence (Chao *et al.*, 2005; English *et al.*, 2004; Larsson *et al.*, 2005). A large-cohort epidemiology study of a US population, aiming at identifying association between red/lean meat variables and incidence of CRC, included unprocessed, barbequed and charred chicken within the frame of investigation. The authors observed a plain inverse relationship between consumed chicken - prepared by any of these cooking schemes - and CRC (Miller *et al.*, 2013). These latter observations thus indirectly support haem as a link in the association between high intake of red meat and an elevated risk of developing CRC, because poultry is comparatively low in haem with levels about one tenth of those typically found in red meat (Bingham *et al.*, 2002).

Nitrosyl-haem is an even more potent inducer of NOC and pre-neoplastic intestinal lesions

Inorganic nitrite, through intestinal conversion of precursor compounds to NOCs, has been proposed as one among the several potential causative agents of food-borne risks of contracting

CRC. Feeding rats with nitrite, at doses well beyond those of human exposure via food or drinking water, did however not produce pre-neoplastic intestinal lesions (Chenni *et al.*, 2013). In many parts of the world chicken meat ï not associated with increased CRC risk - is preserved with nitrite or nitrate (Cross *et al.*, 2010). Moreover, nitrite intake was not correlated with higher CRC risk in a large Finnish epidemiology investigation (Knekt *et al.*, 1999). Neither could a risk of contracting gastric malignancy be attributed to nitrite alone in a study of a cohort in the Netherlands (van Loon *et al.*, 1998). Conversely, processed meat seems to be associated with higher load of NOC and is even more closely related to CRC incidence than non-cured beef or pork (Demeyer *et al.*, 2008; Santarelli *et al.*, 2008). As reported in three early epidemiology reviews the average estimated excess risk was roughly 50 % higher for processed meat, relative to that of fresh meat (Larsson and Wolk, 2006; Norat *et al.*, 2002; Sandhu *et al.*, 2001). More recent research has confirmed a higher risk attached to consumption of processed meat, but with slightly lower inferred figures (Aune *et al.*, 2013; Chan *et al.*, 2011). Moreover, colonic NOC levels in rats on a feed rich in hot dogs was about twice those of subjects fed beef (Mirvish *et al.*, 2003). Indeed, NO from nitrite in contact with haem can form nitrosyl haem (Santarelli *et al.*, 2008). Notably, cooked and oxidized processed meat, compared with less oxidized feed, resulted in higher levels of mucin-depleted foci in experimental rats, thus signifying the highest potency of nitrosyl haem (Santarelli *et al.*, 2010). As further outlined below, the intestinal impact of haem and nitrosyl-haem appears to go beyond the generation of NOCs. Corpet and colleagues have suggested three different routes of haem-/nitrosyl-haem mediated cancerogenicity: i) formation of tumor-promoting haem species in the gut, ii) formation of endogenous N-nitroso substances

and iii) induction of fat peroxidation (in food) (Corpet, 2011). Arguably, each such proposed class of action holds appreciable complexity, involving a range of distinct mechanisms.

RED MEAT OR HAEM-INDUCED CHANGES TO THE CELLULAR MACHINERY

Disruption of proliferative control, apoptosis and development

Using an outbred Wistar rat model a Dutch research group, with A Sesink and R van der Meer in principal positions, reported - already at the turn of the century ï striking hyper-proliferation of the intestinal epithelium, in response to haem-added feeding. Increased gut cytotoxicity also occurred in accordingly fed animals and the growth-promoting action of haem was thus construed as triggered by a compensatory mechanism, induced by haem-mediated toxicity (Sesink *et al.*, 1999; Sesink *et al.*, 2000). Around the same time, aberrant crypt foci in rat colon - including elevated proliferation - were likewise identified in a similar experimental setting by Pierre and colleagues (Pierre *et al.*, 2003). Continued investigation confirmed and advanced on these observations, revealing increased crypt depth in rats fed with haem-added diet as well as altered mRNA expression of several tissue-specific genes of the colon, including an about 10-fold down-regulation of mucosal pentraxin (van der Meer-van Kraaij *et al.*, 2005). The exact function of this intestinal protein is not known, but reduced expression has been observed in experimentally induced pre-cancerous gut lesions (Drew *et al.*, 2005). Further, by means of caspase-3 expression assay and Ki-67 immuno-histochemistry, markedly enhanced colonocyte apoptosis was subsequently observed, jointly with increased cellular proliferation and histologically assessed crypt hyperplasia, in haem-fed rats (de Vogel *et al.*, 2008). More recent *in*

vivo studies, based on histochemical, biochemical and molecular profiling techniques combined, have disclosed intriguing intestinal changes in response to haem-added diet at higher molecular-mechanistic resolution levels. Notably, survivin, a major apoptosis-inhibiting protein, was markedly (3-fold) up-regulated at the intestinal crypt level of the test mice, whereas *TP53* expression was significantly suppressed, thus substantiating earlier observations on haem-induced suppression of gut apoptosis (Ijssennagger *et al.*, 2012; Ijssennagger *et al.*, 2013). Moreover, several development regulation genes, notably *Wif1* and *BMP2* - key genes involved in the fine-tuned differentiation of intestinal stem cells (see above for short overview of cellular functions) - were markedly down-regulated, which likewise applied to IL-15, a cytokine known to inhibit growth of tumor cells (Ijssennagger *et al.*, 2013). A conceptual framework, as proposed by Ijssennagger and colleagues, holds that a (not yet molecularly defined) lipid peroxide derivative of dietary haem triggers compensatory (pre-) malignant development (proliferative, anti-apoptotic and/or developmental-disrupting) largely through cytotoxic action (de Vogel *et al.*, 2005; Ijssennagger *et al.*, 2013).

Arginine: implicated in the diet-CRC association?

Arginine, a natural precursor of polyamines through endogenous enzymatic conversion, has been proposed as a candidate red meat-borne risk factor of CRC. This view appreciates red meat as a source of arginine, ultimately giving rise to a special class of biogenic amines known as polyamines, which are essential aliphatic amines (Zell *et al.*, 2007). Clearly, polyamines are implicated in diverse cellular events, including proliferative stimulation. Putrescine, spermidine and spermine - key physiological polyamines - are provided endogenously from arginine and

ornithine, as accomplished by ornithine decarboxylase (ODC), which catalyzes the initial step in polyamine biosynthesis: conversion of ornithine to putrescine (Pegg, 2009).

A series of clinical or nutritional epidemiology investigations confirm a preventive action of DFMO - an ODC inhibitor - with respect to risk of developing colorectal adenomas and CRC, thus providing support to a functional connection between polyamines and intestinal malignancy (Gerner and Meyskens, 2009; Meyskens *et al.*, 2008; Raj *et al.*, 2013; Vargas *et al.*, 2012). This line of thought gains further credence from observations in experimental models: polyamine biosynthesis is commonly elevated in epithelial tumors and suppression of their endogenous production impedes tumor proliferation (Ignatenko *et al.*, 2011; Ignatenko *et al.*, 2009). Mice heterozygous for a colorectal adenoma-predisposing mutation in *APC* showed increased high-grade colon adenoma incidence, when exposed to high arginine feed (Zell *et al.*, 2007). Moreover, a connection between *c-Myc* - implicated in many malignancies - and polyamines has been demonstrated, i.e. the action of Myc on tumor initiation and progression can be weakened through intercepting endogenous polyamine production (Evageliou and Hogarty, 2009). Further, recent molecular findings link polyamines to the LIN28/let-7 pathway, i.e. they can thus stimulate the high mobility group A2 (HMGA2) factor, being associated with poor survival in colorectal cancer (Paz *et al.*, 2013; Wang *et al.*, 2011). A highly related series of observations pertain to ODC itself: polymorphism in the encoding gene is tied to various risk levels of developing either colorectal adenomas or manifest CRC. Notably, this risk is, at least in part, associated with two common genetic variants, one located within intron 1 involving a G allele (rs2302615) and another situated downstream of the gene (Barry *et al.*, 2011; Hubner *et al.*, 2008).

Polyamines are, however, obtained from diverse dietary sources, either in the de novo form, or in the form of amino acid precursors, or produced by intestinal microorganisms (Larque *et al.*, 2007). According to information available in the Official Danish Food Composition Database, arginine contents in beef or pork commodities are no higher than those in chicken or salmon (DFCD, 2009). Furthermore, polyamines are abundant in various key vegetables and fruits, as typified by corn, red beans, soybean, orange and grapefruit (Atiya Ali *et al.*, 2011). Taken together, arginine seemingly has carcinogenic potential but this direction ï in the context of a model for red/processed meat and risk of contracting CRC ï is nonetheless compounded by an abundant presence of arginine in several other foods as well.

Possible epigenetic modulations

Various micronutrients, e.g. folic acid, lycopene, ascorbic acid and several additional food ingredients, such as genistein and other phytoestrogens, are reportedly able to induce epigenetic changes in mammals (Chen *et al.*, 2013; Duthie, 2011; Guerrero-Bosagna and Skinner, 2014; Kikuno *et al.*, 2008; King-Batoon *et al.*, 2008). As of today, little is known about whether red or processed meat carry such potential and ï should this assumption eventually find experimental support - the ingestion levels that might trigger such changes to the consumerôs intestinal epithelial mucosa. Recently, Hebels and colleagues reported a rich set of alterations in human gut biopsy samples, as revealed by gene expression analysis, following one week on a high red meat diet. One of those changes involved potential activation of a nucleosome remodelling pathway ï more precisely the NuRD complex - which is implicated in methylation-mediated gene silencing (Hebels *et al.*, 2012). Moreover, Ijssennagger and co-workers have identified reduced gene and protein expression of a set of developmental genes in mice, in response to a feed spiked with

haem, thus mimicking a human daily consumption of about 160 g red meat. Two among those (suppressed) genes are *Wif1* and *BMP2*, which both antagonize the Wnt signaling cascade and promotes differentiation of mucosal cells at the crypt-villus border, respectively (Hardwick *et al.*, 2008; Holland *et al.*, 2013; Ijssennagger *et al.*, 2012; Ijssennagger *et al.*, 2013). In colonic mucosa of male CRC patients, significantly higher level of *Wif1* CpG methylation, relative to healthy humans, has been observed (Belshaw *et al.*, 2008). As revealed by investigations on human cell lines *BMP2* is, similar to *Wif1*, silenced through CpG island methylation in many cases (Kodach *et al.*, 2011). In brief, these various observations are far from instructive as to whether haem induces gene silencing via promoter methylation, but nonetheless provide some support to a possible epigenetic involvement in red/processed-mediated intestinal carcinogenesis.

INFLAMMATORY MECHANISMS OF POTENTIAL RELEVANCE TO COLORECTAL ADENOMA AND CRC

General aspects

Chronic inflammation is supposed to be an integrated part of the patho-physiology of many diseases, including some among those of the neoplasm category. Notably, patients with inflammatory bowel diseases (IBD) ï ulcerative colitis and Crohnõs disease - are at higher risk of developing colorectal malignancy, relative those devoid of these disorders. This connection is likewise seen in experimental animals with induced intestinal inflammation (Ullman and Itzkowitz, 2011). Consequently, nutritional-oriented research on CRC has taken on the issue of linking dietary patterns to inflammation. Briefly, the n-6- polyunsaturated fatty acids (PUFAs), relatively abundant in red meat, are reported to promote an inflammatory response through

various mechanisms, whereas n-3 PUFAs are generally perceived as anti-inflammatory. Notably, linolenic acid, an archetype n-6 PUFA, is physiologically converted to arachidonic acid, a precursor of eicosanoids, i.e. prostaglandins, leukotriens and thromboxanes. These compounds are mediators of inflammation in part through stimulating the expression of interleukin (IL-1ƃ) and tumor necrosis factor (TNF)-Ŭin monocytes as well as IL-6 and IL-8 in endothelial cells. Expression levels of TGF-ƃ1 and interferon-ɔ are likewise modulated (Calder, 2008; Harbige, 2003). Conversely, Ŭ-linolenic acid, a principal n-3 PUFA, is converted to eicosapentaenoic acid and docosahexaenoic acid, which are reported to suppress inflammation through a range of pathway interactions, including modification of the eicosanoid set-up (Calder, 2008; Innis and Jacobson, 2007).

Fat fish and oils derived from such species are rich sources of n-3 PUFAs and it has thus been proposed that the observed anti-inflammatory response is largely attributed to these fatty acids, abundantly present in fatty fish, but also cod ï although comparatively low in n-3 PUFAs - has this effect (Ouellet *et al.*, 2008; Pot *et al.*, 2010). Epidemiology investigations on high/low fish consumption in relation to CRC incidence are not fully consistent, but a large European study nonetheless showed an inverse risk association with fish intake (Norat *et al.*, 2005). This observation gains further support, although with borderline significance, from a subsequent meta-analysis encompassing 14 epidemiology investigations (Geelen *et al.*, 2007). A more recent study, rather focused on a positive relationship between red meat and CRC, found a trend towards an independent (inverse risk) impact of fish consumption beyond that related to food substitution alone (Daniel *et al.*, 2011). The n-3-PUFAs and other compounds present in fish could thus possibly account for lower CRC risk through attenuated inflammatory response (in

the intestine), but certain conflicting findings impose consternation on this assumption. Neither of two independent studies of standard *versus* high fish diets did record any appreciable disparity between the test groups, with respect to anyone among several markers of intestine inflammation, including calprotectin (Joosen *et al.*, 2010; Pot *et al.*, 2010). One of those reports, however, observed 25 % lower systemic levels of C-reactive protein (CRP; a biomarker of inflammation) in the fish group, relative to those of controls (Pot *et al.*, 2010). Although systemic CRP is known to correlate with risk of developing several cancer diseases, possibly including those of the intestinal tract, the fish diet-induced inflammatory attenuation does not lend itself to straightforward construal in the context of CRC (Allin and Nordestgaard, 2011; Pot *et al.*, 2010).

Potential relevance to red/processed meat consumption

Red meat is typically rich in saturated fats, n-6 PUFAs and cholesterol. Some reports show that markers of inflammation, particularly C-reactive protein (CRP), are elevated in consumers on a high red meat diet. For instance, significant elevation of CRP with increasing red meat consumption was seen in a cross-sectional study, encompassing nearly 500 individuals (Azadbakht and Esmaillzadeh, 2009). Another similarly designed, but much larger study, likewise concludes on a significant association between CRP and red meat ingestion. This connection also included ɔ-glutamyltransferase (GGT; a marker of oxidative stress) (Montonen *et al.*, 2013). Conversely, an 8-week parallel-designed study, encompassing 60 participants, could not identify higher CRP levels among individuals on a high red meat diet, relative to those on a regular consumption pattern (Hodgson *et al.*, 2007). Further, a much larger investigation, being an excerpt - 3690 individuals - of the US Nurseôs Health Study cohort aimed at assessing

red meat intake and various biomarkers of inflammation. Indeed, regression modeling of the data revealed a correlation of higher plasma CRP with red meat consumption, but this association attenuated to non-significant levels subsequent to adjustment to BMI (Ley *et al.*, 2013). Another investigation likewise reported no association between CRP responses and intake of processed meat (van Woudenbergh *et al.*, 2012). Lastly, neither circulating CRP nor fecal calprotectin were significantly raised in volunteer subjects with increasing consumption of red meat (Joosen *et al.*, 2010).

A well-documented relationship between certain anti-inflammatory drugs and prevention of CRC, jointly with the above-mentioned relationship between IBD and CRC incidence, may indirectly suggest an involvement of inflammation as a mechanism behind the red/processed meat-CRC association. There is ample support in the literature to the notion that low-dose NSAID administration, typified by aspirin or sulindac, can alleviate already diagnosed CRC, including risk of relapse subsequent to therapy, and prevent overt disease among pre-cancerous colorectal adenoma patients (Chan *et al.*, 2009; Cole *et al.*, 2009; Din *et al.*, 2010; Sandler *et al.*, 2003). Up-regulated cyclooxygenase-2 (COX-2), a key target of many NSAIDs, can enhance colorectal cancer progression though poorly understood mechanisms. An implication of inflammation-attenuation in these clinical observations is suggested by lower effect of NSAIDs in CRC-conditions associated with up-regulated COX-2 synthesis and PGE_2 production (Liao *et al.*, 2012; Nishihara *et al.*, 2013). However, other pharmacological actions apart from those involving inflammation strictly, and perhaps even more critical in the context of CRC, are likewise at play here. First, PGE_2 - via binding to the prostaglanding GPRC receptor EP2 - is shown to stimulate transcription of ß-catenin regulated genes by virtue of enhanced

phosphorylation of GSK-3ß, leading to its efficient ubiquitination and subsequent proteasomal degradation, resulting in stabilization of ß-catenin (Castellone *et al.*, 2005). Thus, attenuation of PGE_2 suppresses activation of Wnt/ß-catenin signaling - a cornerstone of colorectal carcinogenesis (Polakis, 2012). Second, a series of in-depth experimental reports by Zhang and co-workers has identified apoptosis as a key mechanism of NSAIDs on various colorectal cancer cells. Using sulindac and non-modified as well as genetically engineered variants of the colorectal carcinoma cell line HCT116, the research group showed that SMAC (second mitochondria-derived activator of caspase) is central to the NSAID-mediated apoptotic action (Bank *et al.*, 2008; Kohli *et al.*, 2004). Further, sulindac-induced SMAC-dependent apoptosis was shown to specifically eliminate early malignant intestinal stem cells in $APC^{Min/+}$ mice (Qiu *et al.*, 2010). Hence, the ability of NSAIDs to either suppress proliferation or induce apoptosis in cell lines seems quite independent of their interference with (constitutively expressed) COX-1 or (inducible) COX-2 (Aggarwal *et al.*, 2000; Vogt *et al.*, 2001). Several recent reports by Piazza and colleagues elaborate further on this topic and convincingly demonstrate that the inhibition of malignant colon cell growth by various NSAIDs is not primarily related to COX-2 suppression, but largely depend on various other mechanisms of which inhibition of phosphodiesterase 5 (PDE5) takes a forward position. Thus, the enzymatic intervention attenuates degradation of cGMP, thereby activating protein kinase G (PKG), which ultimately down-regulates ß-catenin mediated transcription (Li *et al.*, 2013; Tinsley *et al.*, 2010). Furthermore, NSAIDs are known to promote polyamine catabolism, thereby easing a proliferative load on gut epithelium. A combined-drug preventive therapy regimen, involving sulindac and an ODC inhibitor (DFMA), is seemingly more efficaous than either drug alone, thus suggesting an involvement of additional

factors, notably polyamines (Meyskens *et al.*, 2008; Raj *et al.*, 2013). Analogous with results from CRC-preventive therapy with NSAID, individuals at risk of developing sporadic colorectal adenomas and featuring high polyamine intake, are at less benefit from the combination therapy, relative to those of intermediate consumption (Raj *et al.*, 2013). Lastly, patients on chronic therapy with entirely distinct drug classes, e.g. selective serotonin reuptake inhibitors (SSRIs) or simvastatin-type cholesterol-lowering pharmaceuticals, are likewise at significantly lower risk of developing colorectal cancer (Kodach *et al.*, 2007; Xu *et al.*, 2006).

Lipid peroxidation products, possibly also including certain oxysterols, produced in animal feeding studies of red meat or purified haem/nitrosyl haem, are known to activate several components of the inflammatory machinery, such as COX-2 and NF-əB (Gueraud *et al.*, 2010). Moreover, a core cytokine of inflammatory processes ï IL-6 - is reportedly implicated in the development of CRC (Waldner *et al.*, 2012). Recent exhaustive research has demonstrated that NF-əB activation strongly promotes Wnt signaling through enhanced ß-catenin-mediated transcription, thereby inducing de-differentiation of colonic villus cells to aberrantly positioned crypt cells. This finding suggest an implication of NF-əB in the development of colorectal cancer (Schwitalla *et al.*, 2013). Although acting as a key regulator of inflammation, activation of NF-əB does, however, not consistently entail extensive inflammation (Guma *et al.*, 2011).

To summarize, the accumulated scientific documentation is far from consistent with respect to a potential involvement of inflammatory processes in general as an underlying mechanism of the significant association between high red/processed meat consumption and an elevated risk of contracting CRC, but nonetheless tends to disfavor this tenet. Seemingly, however, interaction of the food component(s) with certain specific endogenous components of the inflammatory

pathway setup, such as anyone or several among IL-6, COX-2 and PGE2, either directly or circuitously, is potentially part of red meat and processed meat-induced colorectal carcinogenesis.

FAT OXIDATION PRODUCTS AS POTENTIALLY IMPLICATED IN CRC

Numerous studies have highlighted the role played by unbalanced, excessive consumption of animal omega-6 fatty acids, e.g. linoleic and arachidonic acid, in the pathogenesis of chronic diseases (Chao *et al.*, 2005; Simopoulos, 2006). Notably, excessive dietary intake of cholesterol and n-6 polyunsaturated fatty acids (PUFAs) present in animal fats has been tentatively associated with increased risk of colorectal cancer (Pot *et al.*, 2008; Reddy, 2004). Lipids represent one of the most important cellular targets of oxidative stress and arachidonic acid is a selective substrate for non-enzymatic oxidation breakdown of lipids in cellular membranes, known as lipid peroxidation. This peroxidation leads to the formation of various molecules with toxic and mutagenic effects, O^6-CMG adducts being an outstanding instance of the latter type (Lewin *et al.*, 2006; Moonen *et al.*, 2004). Oxidation products of lipids of both exogenous and endogenous origin thus become available at the level of the colonic mucosa. Animal fat, present in red/processed meat, has been proposed as a potential causative agent in the context of CRC development. Although experimental investigations are not fully consistent on this issue, a meta-analysis on clinical epidemiology studies failed to support the notion of fat as an important factor in CRC (Alexander *et al.*, 2009). Haem iron is, though, known to produce a range of epoxides and aldehydes, which are perceived as risk factors. Malondialdehyde (MDA) and 4-HNE are such agents, of which the latter is a major toxic lipid peroxidation compound and associated with

interference with various signal transduction pathways (Bastide *et al.*, 2011; Kanner, 2007) [Figure 2].

4-HNE is a marker of lipid peroxidation and increases in proportion to ingested haem, thus signifying a tight connection between haem and the formation of such oxidation products (Pierre *et al.*, 2006). This aldehyde was shown to trigger apoptosis in normal endothelial cells, but induced only a weak such response in premalignant cells, thus providing a growth advantage to the latter type (Pierre *et al.*, 2007). Moreover, 4-HNE can interfere with the expression of major developmental genes, such as *Wnt* and *Hh*. A connection between 4-HNE and Wnt activation was shown by Liu and colleagues (Liu *et al.*, 2013). MDA is likewise reported to stimulate Wnt expression (Wang *et al.*, 2014). With a view to actions of certain lipid peroxidation products on the aforementioned cellular components ï in the context of substance-induced cellular flow between normal and malignant epithelial stem cells - these and related oxidized lipids may be perceived as candidate propellants in the direction towards (cancer) stem cells (Iliopoulos *et al.*, 2011; Schwitalla *et al.*, 2013).

Oxysterols are the products of the oxidation of cholesterol, which is generated by means of enzymatic reactions by certain members of the cytochrome P450 family of enzymes or through non-enzymatic reactions involving various reactive compounds (Chang *et al.*, 2006). Although various oxysterols play different regulatory roles in normal cellular processes, such as cholesterol homeostasis and immunity, this class of substances is also implicated in multiple disease states, including atherosclerosis and macular degeneration (Brown and Jessup, 1999; Rodriguez and Larrayoz, 2010; Spann and Glass, 2013). Numerous reports show that certain oxysterols are likewise associated with an array of distinct cancer diseases, including those of the

colon, lung, skin, breast cancer and bile duct (de Weille *et al.*, 2013; Vejux and Lizard, 2009; Yoon *et al.*, 2004). The underlying mechanisms are not fully understood but certain observations may shed some light on possible critical cellular events. For instance, some oxysterol-derivatives, such as cholesterol-epoxide and cholestanetriol, have been attributed to mutagenic and genotoxic actions, as revealed by results from human colorectal-derived cell line (Biasi *et al.*, 2013). Other oxysterols are associated with the induction of pro-inflammatory cytokines and chemokines, notably interleukin (IL)-1ϐ and IL-8, which might contribute to the development of colorectal malignancy. This likewise applies to cyclooxygenase-2 expression, a key pro-inflammatory protein, but also broadly implicated in the promotion of cellular growth and apoptosis inhibition (Trifan and Hla, 2003). Inflammatory actions may also be mediated through the activation of liver X receptor nuclear receptors (LXRŬand LXRß) for oxysterols (Edwards *et al.*, 2002). There are, however, conflicting consequences of oxysterol interaction with the LXR receptors in the context of CRC initiation or promotion. Notably, ligands of LXRs are reported to suppress the activity of either wild-type or mutant ß-catenin, a major component of the Wnt/ß-catenin signaling cascade and highly implicated in CRC, in malignant colorectal cells (Uno *et al.*, 2009).

Apart from several broadly painted oxysterol actions, as briefly outlined above, a few more specific effects have been suggested as potentially implicated in colorectal malignancy. Notably, certain oxysterol species can enhance protein expression of TGF-ϐ1 in human epithelial and other cell types. Although TGF-ϐ1 is generally associated with epithelial growth arrest and differentiation-promotion of crypt-located stem cells to enterocytes, an involvement in colorectal carcinogenesis has nonetheless been proposed (Biasi *et al.*, 2008; Radtke *et al.*, 2006). According

to this tenet, TGF-β1 conveys a selective advantage to the malignant gut epithelial cells in an intermediate phase of disease progression, which are initially often receptive to the factorôs anti-proliferative signal, although this type of regulation is lost over time. Thus, enhanced TGF-β signaling is thought to selectively favor malignant cell clones that are insensitive to the cytokine (Biasi *et al.*, 2008). Yet another proposed tumor promoting involvement of TGF-ß pertains to its interference with the tumor microenvironment: Jointly with IL-6, TGF-β stimulates TH_{17} lymphocytes, which are highly conducive to the establishment of a tumor-supportive stroma (Grivennikov *et al.*, 2010; Yu *et al.*, 2009).

Another, perhaps even more intriguing candidate implication of oxysterol in colorectal carcinogenesis, is rooted in the selective binding of certain such species to the Smoothened (Smo) transmembrane receptor of the Hedgehog signaling cascade (Dwyer *et al.*, 2007). More specifically, 20 (S) hydroxyl cholesterol [20 (S) OHC] was shown to behave as a competitive allosteric activator of Smo (Nachtergaele *et al.*, 2012). A subsequent investigation extended these observations to 7-keto-25 hydroxy cholesterol (7-keto-25 OHC) and 7-keto-27-OHC and concluded that these oxysterols, as well as 20 (S) OHC, interact directly with a conserved extracellular cysteine-rich domain of Smo (Myers *et al.*, 2013). This observation holds a special potential, owing to the Hedgehog pathwayôs deep involvement in embryonic development and the regulation of normal stem cell as well as cancer stem cells alike (Barakat *et al.*, 2010; Marini *et al.*, 2011). Notably, cell lines derived from human CRC biopsies showed expression of the Hedgehog cascade genes, i.e. SHH (encodes Sonic Hedgehog) and the Gli family of transcription factors. Actually, this signature was even more pronounced in $CD133^+$ metastatic cell clones, suggesting a connection to tumor migration and re-colonisation (Varnat *et al.*, 2009).

Furthermore, activated Smo can stimulate ß-catenin dependent Wnt signaling, as demonstrated both *in vitro* and in a murine model (Arimura *et al.*, 2009). Assuming that haem or nitrosyl haem can stimulate the conversion of cholesterol to anyone among the Smo-activating species, this chain of connected cues ï i.e. oxysterols, the Hedgehog and Wnt/ß-catenin pathways - is thus of potential relevance to the red/processed meat issue. A connection to Wnt/ß-catenin is here of particular interest, because aberrant activation of this key pathway is almost consistently seen in CRC (Vermeulen *et al.*, 2010; White *et al.*, 2012).

CLOSING COMMENTS: DEVELOPMENT OF CRC AND THE POTENTIAL ASSOCIATION TO FACTORS IN RED AND PROCESSED MEAT

Several mechanisms behind and the association between red/processed meat and CRC

Meta-analyses of nutrition epidemiology data reveal a dose-dependent relationship between ingested red/processed meat and the risk of contracting CRC (Aune *et al.*, 2013; Chan *et al.*, 2011; WCRF/AICR, 2007). This observation lends further support by a dose-effect relationship in experimental animals fed red meat, with respect to pre-cancerous lesions (Bingham *et al.*, 2002; Hughes *et al.*, 2001; Pierre *et al.*, 2004; Pierre *et al.*, 2006). Available experimental data do, however, not converge on any acceptable single mechanism that alone can explain how red and processed meat act in the development of CRC. Seemingly, several diverse factors combined, of which some are outlined above, are needed for meat-induced intestinal tumor development. Although mutagenic mechanisms are presumed as instrumental to the carcinogenesis process, either through direct oxidative insult or via lipid peroxidation, more recent findings show that other mechanisms likewise have to be seriously considered (Hebels *et*

al., 2012; Ijssennagger *et al.*, 2012; Joosen *et al.*, 2009; Joosen *et al.*, 2010; Miller *et al.*, 2013). The accumulated literature in this area suggests that red/processed meat-induced CRC sits on a multi-factorial foundation at both cellular and molecular levels [Figure 3]. Meanwile, a large volume of reports converge on haem and nitrosyl haem, abundant in red/processed meat contrary to fish, chicken and other white meat, as major agents underlying the association between red/processed meat and CRC (Bastide *et al.*, 2011). Furthermore, nitrosyl haem, which occurs at higher levels in processed meat, appears to possess even higher propensity to induce pre-neoplastic colonic lesions, relative to that of haem (Bastide *et al.*, 2011).

Inflammation, lipid peroxidation and cholesterol oxidation

Animal feeding studies of red meat or purified haem/nitrosyl haem reveal that lipid peroxidation products, possibly including certain oxysterols, can activate several components of the inflammatory machinery, such as COX-2 and NF-əB (Gueraud *et al.*, 2010). In some disparity with these findings, histopathological and molecular investigation of intestinal mucosa of mice fed a haem-added diet showed no sign of inflammatory response (Ijssennagger *et al.*, 2012). Overall, the current literature within clinical nutrition and *in vivo* experimental food research, although not consistent, provides little support to the assumption that inflammation broadly is central to the aforementioned diet-disease association (Joosen *et al.*, 2010; Ley *et al.*, 2013; van Woudenbergh *et al.*, 2012).

Lipid peroxidation triggers the formation of various reactive and bioactive agents, such as alkanes, aldehydes and isoprostanes (Corpet, 2011; Dwivedi *et al.*, 2007). A preferential induction of apoptosis by 4-HNE in healthy colorectal epithelial cells, relative to premalignant counterparts, may favor progression of neoplastic alterations (Pierre *et al.*, 2007). Non-enzymatic

conversion of cholesterol, either mediated directly by haem/nitrosyl haem or via NOC intermediates to bioactive oxysterols is, however, not widely appreciated in the literature as a candidate mechanism of red/processed meat-induced carcinogenesis. Findings *in vitro* on oxysterol-mediated - e.g. 20 (S) OHC - activation of Smo of the Hedgehog signaling cascade constitute a sequence of molecular events potentially leading to malignancy (Corcoran and Scott, 2006; Myers *et al.*, 2013; Nachtergaele *et al.*, 2012). Canonical Hedgehog signaling is essential in its own right to growth and metastasis of colon epithelial neoplastic cells, as shown in human malignant colonic tissue (Oniscu *et al.*, 2004; Varnat *et al.*, 2009). There is, however, also a connection to the ï in this context ï more recognized and fundamentally important Wnt cascade: active Smo is able to trigger Wnt signaling in intestinal tumor cells through a non-canonical pathway, thus giving rise to polyp adenoma epithelial cells *in vivo* (Arimura *et al.*, 2009). It remains, however, to assess whether haem/nitrosyl haem, either alone or through NOCs, can convert cholesterol to Smo-binding oxysterols specifically.

Other potential routes of perturbing developmental pathways, in response to red/processed meat ingestion

The identification of Notch and Wnt as targeted pathways, as revealed by gene expression analysis of intestinal biopsies from volunteers on a high red meat diet, is yet another support to the potential involvement of genes critical to the development of carcinogenesis (Hebels *et al.*, 2012). Down-regulation of a Wnt pathway inhibitor and other members of developmental cascades - commonly disrupted in CRC - in response to haem likewise brings these key cellular regulators to the surface, in the context of red/processed meat-induced tumorigenesis (Ijssennagger *et al.*, 2012). Moreover, the aforementioned experimental *in vivo* setting revealed

concurrent appearance of haem-induced intestinal cytotoxicity and an elevated epithelial proliferation. The authors thus proposed a haem-induced (pre-) tumorigenesis model, in which the intestinal epithelial proliferative induction occurs as a compensatory response to haem-mediated cytotoxicity (Ijssennagger *et al.*, 2013). Further, a few observations convey indirect support to a possible involvement of epigenetic mechanisms in the red meat issue, especially the NuRD complex (Hebels *et al.*, 2012). Lastly, whether partly degraded red meat protein fragments and/or peptides formed by digestion and/or microbial activity in the gastrointestinal tract - in response to the risky diet - could act as growth promoting factors for malignant cells in the colorectum likewise need further investigation.

Why has processed meat, relative to red meat, seemingly higher potency of inducing CRC development?

Processed meat, of which a sizable fraction is cured with sodium nitrite and thereby contains nitrosyl-haem, appears to be associated with a higher load of NOCs and is even more closely associated to an increased CRC incidence than non-cured beef or pork (Demeyer *et al.*, 2008; Santarelli *et al.*, 2008). Markedly higher oxidative capacity of nitrosyl-haem over haem might contribute to the suggested difference in potency between processed meat and red meat to trigger CRC development, but various additional or even alternative mechanisms remain viable options (Ijssennagger *et al.*, 2012; Santarelli *et al.*, 2008). A fundamental issue is whether nitrosyl haem simply carries higher bioactivity across the entire spectrum of effects, but otherwise being equivalent to haem, or whether it possesses certain unique feature(s) that induce development of CRC by additional or partly non-overlapping gene mutation or signal transduction disruption.

Notably, the higher risk ratios observed for processed meat may not solely relate to sodium nitrite treated food items specifically, thus imposing additional complexity on the field.

PROPOSED REMEDIES TO THE RED/PROCESSED MEAT-CRC CONNECTION

Besides reduced processed meat consumption, an alternative route to alleviate the risk of contracting diet-related CRC might involve meat preservation techniques not based on sodium nitrate; such alternatives are indeed available. One of those uses an adapted hurdle technology, which is reported to prevent growth of *C. sporogenes*, *S. aureus* and *B. cereus* over three months (Chawla and Chander, 2004). Another proposed option, which does not need nitrite replacement, emerges from an inhibitory influence of calcium on haem-induced aberrant colonic crypts in mice, suggestively through precipitation of haem (Pierre *et al.*, 2003). Subsequent research has reinforced this finding (Allam *et al.*, 2011; Pierre *et al.*, 2008). However, calcium phosphate raised the level of pre-neoplastic changes in control animals, whereas calcium carbonate proved devoid of this untoward action, thus seemingly being a viable option for the food industry (Allam *et al.*, 2011; Pierre *et al.*, 2008).

Calcium carbonate is already an approved food additive, but as yet without grant for processed or other red meat products (CAC, 2013). Assuming that calcium carbonate eventually receives regulatory endorsement for meat curing, jointly with nitrite, based on an ability to safely inactivate haem/nitrosyl-haem, seemingly the main first-line culprits of the red/processed meat-CRC risk connection, it would thus be licensed largely to neutralize an indirectly operating detrimental effect of another preservation agent. The above-mentioned rationale for introducing

yet another food additive broadly into the same food category might thus induce consternation among some consumers. Anyway, it is perhaps timely for the food industry to adopt practices analogous with those of certain pharmacotherapy areas ï mitigation of potential adverse effects of a drug by means of other drug(s).

REFERENCES

Aggarwal, S., Taneja, N., Lin, L.*, et al.* (2000). Indomethacin-induced apoptosis in esophageal adenocarcinoma cells involves upregulation of Bax and translocation of mitochondrial cytochrome C independent of COX-2 expression. *Neoplasia,* **2 :** 346-356.

Al-Sohaily, S., Biankin, A., Leong, R.*, et al.* (2012). Molecular pathways in colorectal cancer. *J Gastroenterol Hepatol,* **27 :** 1423-1431.

Alexander, D. D., Cushing, C. A., Lowe, K. A.*, et al.* (2009). Meta-analysis of animal fat or animal protein intake and colorectal cancer. *Am J Clin Nutr,* **89 :** 1402-1409.

Allam, O., Bahuaud, D., Tache, S.*, et al.* (2011). Calcium carbonate suppresses haem toxicity markers without calcium phosphate side effects on colon carcinogenesis. *Br J Nutr,* **105 :** 384-392.

Allin, K. H. and Nordestgaard, B. G. (2011). Elevated C-reactive protein in the diagnosis, prognosis, and cause of cancer. *Crit Rev Clin Lab Sci,* **48 :** 155-170.

Arimura, S., Matsunaga, A., Kitamura, T.*, et al.* (2009). Reduced level of smoothened suppresses intestinal tumorigenesis by down-regulation of Wnt signaling. *Gastroenterology,* **137 :** 629-638.

Armaghany, T., Wilson, J. D., Chu, Q.*, et al.* (2012). Genetic alterations in colorectal cancer. *Gastrointest Cancer Res,* **5 :** 19-27.

Asangani, I. A., Rasheed, S. A., Nikolova, D. A.*, et al.* (2008). MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pdcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. *Oncogene,* **27 :** 2128-2136.

Ashktorab, H., Schaffer, A. A., Daremipouran, M.*, et al.* (2010). Distinct genetic alterations in colorectal cancer. *PLoS ONE,* **5 :** e8879.

Ashley, N. (2013). Regulation of intestinal cancer stem cells. *Cancer Lett,* **338 :** 120-126.

Atiya Ali, M., Poortvliet, E., Stromberg, R.*, et al.* (2011). Polyamines in foods: development of a food database. *Food Nutr Res,* **55 :** 5572.

Aune, D., Chan, D. S., Vieira, A. R.*, et al.* (2013). Red and processed meat intake and risk of colorectal adenomas: a systematic review and meta-analysis of epidemiological studies. *Cancer Causes Control,* **24 :** 611-627.

Azadbakht, L. and Esmaillzadeh, A. (2009). Red meat intake is associated with metabolic syndrome and the plasma C-reactive protein concentration in women. *J Nutr,* **139 :** 335-339.

Balaguer, F., Link, A., Lozano, J. J.*, et al.* (2010). Epigenetic silencing of miR-137 is an early event in colorectal carcinogenesis. *Cancer Res,* **70 :** 6609-6618.

Bank, A., Wang, P., Du, C.*, et al.* (2008). SMAC mimetics sensitize nonsteroidal anti-inflammatory drug-induced apoptosis by promoting caspase-3-mediated cytochrome c release. *Cancer Res,* **68 :** 276-284.

Barakat, M. T., Humke, E. W. and Scott, M. P. (2010). Learning from Jekyll to control Hyde: Hedgehog signaling in development and cancer. *Trends Mol Med,* **16 :** 337-348.

Barker, N., Ridgway, R. A., van Es, J. H.*, et al.* (2009). Crypt stem cells as the cells-of-origin of intestinal cancer. *Nature,* **457 :** 608-611.

Barry, E. L., Mott, L. A., Sandler, R. S.*, et al.* (2011). Variants downstream of the ornithine decarboxylase gene influence risk of colorectal adenoma and aspirin chemoprevention. *Cancer Prev Res (Phila),* **4 :** 2072-2082.

Bastide, N. M., Pierre, F. H. and Corpet, D. E. (2011). Heme iron from meat and risk of colorectal cancer: a meta-analysis and a review of the mechanisms involved. *Cancer Prev Res (Phila),* **4,** 177-184.

Belshaw, N. J., Elliott, G. O., Foxall, R. J.*, et al.* (2008). Profiling CpG island field methylation in both morphologically normal and neoplastic human colonic mucosa. *Br J Cancer,* **99 :** 136-142.

Biasi, F., Chiarpotto, E., Sottero, B.*, et al.* (2013). Evidence of cell damage induced by major components of a diet-compatible mixture of oxysterols in human colon cancer CaCo-2 cell line. *Biochimie,* **95 :** 632-640.

Biasi, F., Mascia, C. and Poli, G. (2008). The contribution of animal fat oxidation products to colon carcinogenesis, through modulation of TGF-beta1 signaling. *Carcinogenesis,* **29 :** 890-894.

Binder, H. J. (2009). *Organization of the gastrointesttinal system*. Saunders, Philadelphia.

Bingham, S. A., Hughes, R. and Cross, A. J. (2002). Effect of white versus red meat on endogenous N-nitrosation in the human colon and further evidence of a dose response. *J Nutr,* **132 :** 3522S-3525S.

Brown, A. J. and Jessup, W. (1999). Oxysterols and atherosclerosis. *Atherosclerosis,* **142 :** 1-28.

CAC. (2013). Codex general standard for food additives - CODEX STAN 192-1995, Codex Alimentarius Commission, pp. 1-332.

Calder, P. C. (2008). Polyunsaturated fatty acids, inflammatory processes and inflammatory bowel diseases. *Mol Nutr Food Res,* **52 :** 885-897.

Castellone, M. D., Teramoto, H., Williams, B. O.*, et al.* (2005). Prostaglandin E2 promotes colon cancer cell growth through a Gs-axin-beta-catenin signaling axis. *Science,* **310 :** 1504-1510.

Center, M. M., Jemal, A., Smith, R. A.*, et al.* (2009). Worldwide variations in colorectal cancer. *CA Cancer J Clin,* **59 :** 366-378.

Chan, A. T., Ogino, S. and Fuchs, C. S. (2009). Aspirin use and survival after diagnosis of colorectal cancer. *JAMA,* **302 :** 649-658.

Chan, D. S., Lau, R., Aune, D.*, et al.* (2011). Red and processed meat and colorectal cancer incidence: meta-analysis of prospective studies. *PLoS ONE,* **6 :** e20456.

Chang, T. Y., Chang, C. C., Ohgami, N.*, et al.* (2006). Cholesterol sensing, trafficking, and esterification. *Annu Rev Cell Dev Biol,* **22 :** 129-157.

Chao, A., Thun, M. J., Connell, C. J.*, et al.* (2005). Meat consumption and risk of colorectal cancer. *JAMA,* **293 :** 172-182.

Chawla, S. P. and Chander, R. (2004). Microbiological safety of shelf-stable meat products prepared by employing hurdle technology. *Food Control,* **15 :** 559-563.

Chen, J., Guo, L., Zhang, L.*, et al.* (2013). Vitamin C modulates TET1 function during somatic cell reprogramming. *Nat Genet,* **45 :** 1504-1509.

Cheng, X. and Blumenthal, R. M. (2010). Coordinated chromatin control: structural and functional linkage of DNA and histone methylation. *Biochemistry,* **49 :** 2999-3008.

Chenni, F. Z., Tache, S., Naud, N.*, et al.* (2013). Heme-induced biomarkers associated with red meat promotion of colon cancer are not modulated by the intake of nitrite. *Nutr Cancer,* **65 :** 227-233.

Cole, B. F., Logan, R. F., Halabi, S.*, et al.* (2009). Aspirin for the chemoprevention of colorectal adenomas: meta-analysis of the randomized trials. *J Natl Cancer Inst,* **101 :** 256-266.

Corcoran, R. B. and Scott, M. P. (2006). Oxysterols stimulate Sonic hedgehog signal transduction and proliferation of medulloblastoma cells. *Proc Natl Acad Sci U S A,* **103 :** 8408-8413.

Corpet, D. E. (2011). Red meat and colon cancer: should we become vegetarians, or can we make meat safer? *Meat Sci,* **89 :** 310-316.

Corpet, D. E. (2012). Mécanismes de lœffet pro-cancer des viandes : revue bibliographique. *Revue Méd Vét,* **163 :** 43-48.

Cross, A. J., Ferrucci, L. M., Risch, A.*, et al.* (2010). A large prospective study of meat consumption and colorectal cancer risk: an investigation of potential mechanisms underlying this association. *Cancer Res,* **70 :** 2406-2414.

Cross, A. J., Greetham, H. L., Pollock, J. R.*, et al.* (2006). Variability in fecal water genotoxicity, determined using the Comet assay, is independent of endogenous N-nitroso compound formation attributed to red meat consumption. *Environ Mol Mutagen,* **47:** 179-184.

Cross, A. J., Leitzmann, M. F., Gail, M. H.*, et al.* (2007). A prospective study of red and processed meat intake in relation to cancer risk. *PLoS Med,* **4 :** e325.

Cross, A. J., Pollock, J. R. and Bingham, S. A. (2003). Haem, not protein or inorganic iron, is responsible for endogenous intestinal N-nitrosation arising from red meat. *Cancer Res,* **63 :** 2358-2360.

Daniel, C. R., Cross, A. J., Graubard, B. I.*, et al.* (2011). Prospective investigation of poultry and fish intake in relation to cancer risk. *Cancer Prev Res (Phila),* **4 :** 1903-1911.

De Roock, W., De Vriendt, V., Normanno, N.*, et al.* (2011). KRAS, BRAF, PIK3CA, and PTEN mutations: implications for targeted therapies in metastatic colorectal cancer. *Lancet Oncol,* **12 :** 594-603.

de Weille, J., Fabre, C. and Bakalara, N. (2013). Oxysterols in cancer cell proliferation and death. *Biochem Pharmacol,* **86 :** 154-160.

de Vogel, J., Jonker-Termont, D. S., van Lieshout, E. M.*, et al.* (2005). Green vegetables, red meat and colon cancer: chlorophyll prevents the cytotoxic and hyperproliferative effects of haem in rat colon. *Carcinogenesis,* **26 :** 387-393.

de Vogel, J., van-Eck, W. B., Sesink, A. L.*, et al.* (2008). Dietary heme injures surface epithelium resulting in hyperproliferation, inhibition of apoptosis and crypt hyperplasia in rat colon. *Carcinogenesis,* **29 :** 398-403.

Demeyer, D., Honikel, K. and De Smet, S. (2008). The World Cancer Research Fund report 2007: A challenge for the meat processing industry. *Meat Sci,* **80 :** 953-959.

Deng, G., Kakar, S. and Kim, Y. S. (2011). MicroRNA-124a and microRNA-34b/c are frequently methylated in all histological types of colorectal cancer and polyps, and in the adjacent normal mucosa. *Oncol Lett,* **2 :** 175-180.

DFCD. (2009). The Official Danish Food Composition Database, National Food Institute/Technical University of Denmark (DTU).

Din, F. V., Theodoratou, E., Farrington, S. M.*, et al.* (2010). Effect of aspirin and NSAIDs on risk and survival from colorectal cancer. *Gut,* **59 :** 1670-1679.

Drew, J. E., Rucklidge, G. J., Duncan, G.*, et al.* (2005). A proteomics approach to identify changes in protein profiles in pre-cancerous colon. *Biochem Biophys Res Commun,* **330 :** 81-87.

Duthie, S. J. (2011). Folate and cancer: how DNA damage, repair and methylation impact on colon carcinogenesis. *J Inherit Metab Dis,* **34 :** 101-109.

Dwivedi, S., Sharma, A., Patrick, B.*, et al.* (2007). Role of 4-hydroxynonenal and its metabolites in signaling. *Redox Rep,* **12 :** 4-10.

Dwyer, J. R., Sever, N., Carlson, M.*, et al.* (2007). Oxysterols are novel activators of the hedgehog signaling pathway in pluripotent mesenchymal cells. *J Biol Chem,* **282 :** 8959-8968.

Edberg, A., Soeria-Atmadja, D., Bergman Laurila, J.*, et al.* (2012). Assessing relative bioactivity of chemical substances using quantitative molecular network topology analysis. *J Chem Inf Model,* **52 :** 1238-1249.

Edwards, P. A., Kennedy, M. A. and Mak, P. A. (2002). LXRs; oxysterol-activated nuclear receptors that regulate genes controlling lipid homeostasis. *Vascul Pharmacol,* **38 :** 249-256.

English, D. R., MacInnis, R. J., Hodge, A. M.*, et al.* (2004). Red meat, chicken, and fish consumption and risk of colorectal cancer. *Cancer Epidemiol Biomarkers Prev,* **13 :** 1509-1514.

Esteller, M., Sparks, A., Toyota, M.*, et al.* (2000). Analysis of adenomatous polyposis coli promoter hypermethylation in human cancer. *Cancer Res,* **60 :** 4366-4371.

Evageliou, N. F. and Hogarty, M. D. (2009). Disrupting polyamine homeostasis as a therapeutic strategy for neuroblastoma. *Clin Cancer Res,* **15 :** 5956-5961.

Fearon, E. R. (2011). Molecular genetics of colorectal cancer. *Annu Rev Pathol,* **6 :** 479-507.

Fearon, E. R. and Vogelstein, B. (1990). A genetic model for colorectal tumorigenesis. *Cell,* **61 :** 759-767.

Feinberg, A. P., Gehrke, C. W., Kuo, K. C.*, et al.* (1988). Reduced genomic 5-methylcytosine content in human colonic neoplasia. *Cancer Res,* **48 :** 1159-1161.

Feng, Z., Hu, W., Amin, S.*, et al.* (2003). Mutational spectrum and genotoxicity of the major lipid peroxidation product, trans-4-hydroxy-2-nonenal, induced DNA adducts in nucleotide excision repair-proficient and -deficient human cells. *Biochemistry,* **42 :** 7848-7854.

Ferlay, J., Steliarova-Foucher, E., Lortet-Tieulent, J.*, et al.* (2013). Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. *Eur J Cancer,* **49 :** 1374-1403.

Fleming, M., Ravula, S., Tatishchev, S. F.*, et al.* (2012). Colorectal carcinoma: Pathologic aspects. *J Gastrointest Oncol,* **3 :** 153-173.

Fodde, R., Smits, R. and Clevers, H. (2001). APC, signal transduction and genetic instability in colorectal cancer. *Nat Rev Cancer,* **1 :** 55-67.

Fre, S., Pallavi, S. K., Huyghe, M.*, et al.* (2009). Notch and Wnt signals cooperatively control cell proliferation and tumorigenesis in the intestine. *Proc Natl Acad Sci U S A,* **106 :** 6309-6314.

Fultz, K. E. and Gerner, E. W. (2002). APC-dependent regulation of ornithine decarboxylase in human colon tumor cells. *Mol Carcinog,* **34 :** 10-18.

Fung, T., Hu, F. B., Fuchs, C.*, et al.* (2003). Major dietary patterns and the risk of colorectal cancer in women. *Arch Intern Med,* **163 :** 309-314.

Geelen, A., Schouten, J. M., Kamphuis, C., *et al.* (2007). Fish consumption, n-3 fatty acids, and colorectal cancer: a meta-analysis of prospective cohort studies. *Am J Epidemiol,* **166 :** 1116-1125.

Gerner, E. W. and Meyskens, F. L., Jr. (2009). Combination chemoprevention for colon cancer targeting polyamine synthesis and inflammation. *Clin Cancer Res,* **15 :** 758-761.

Gottschalg, E., Scott, G. B., Burns, P. A., *et al.* (2007). Potassium diazoacetate-induced p53 mutations in vitro in relation to formation of O6-carboxymethyl- and O6-methyl-2'-deoxyguanosine DNA adducts: relevance for gastrointestinal cancer. *Carcinogenesis,* **28 :** 356-362.

Gratz, S. W., Wallace, R. J. and El-Nezami, H. S. (2011). Recent Perspectives on the Relations between Fecal Mutagenicity, Genotoxicity, and Diet. *Front Pharmacol,* **2 :** 4.

Grivennikov, S. I., Greten, F. R. and Karin, M. (2010). Immunity, inflammation, and cancer. *Cell,* **140 :** 883-899.

Gueraud, F., Atalay, M., Bresgen, N., *et al.* (2010). Chemistry and biochemistry of lipid peroxidation products. *Free Radic Res,* **44 :** 1098-1124.

Guerrero-Bosagna, C. M. and Skinner, M. K. (2014). Environmental epigenetics and phytoestrogen/phytochemical exposures. *J Steroid Biochem Mol Biol,* **139 :** 270-276.

Guma, M., Stepniak, D., Shaked, H., *et al.* (2011). Constitutive intestinal NF-kappaB does not trigger destructive inflammation unless accompanied by MAPK activation. *J Exp Med,* **208 :** 1889-1900.

Hammerling, U., Tallsjo, A., Grafstrom, R., *et al.* (2009). Comparative hazard characterization in food toxicology. *Crit Rev Food Sci Nutr,* **49 :** 626-669.

Hanahan, D. and Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell,* **144 :** 646-674.

Harbige, L. S. (2003). Fatty acids, the immune response, and autoimmunity: a question of n-6 essentiality and the balance between n-6 and n-3. *Lipids,* **38 :** 323-341.

Hardwick, J. C., Kodach, L. L., Offerhaus, G. J.*, et al.* (2008). Bone morphogenetic protein signalling in colorectal cancer. *Nat Rev Cancer,* **8 :** 806-812.

Hebels, D. G., Jennen, D. G., Kleinjans, J. C.*, et al.* (2009). Molecular signatures of N-nitroso compounds in Caco-2 cells: implications for colon carcinogenesis. *Toxicol Sci,* **108 :** 290-300.

Hebels, D. G., Sveje, K. M., de Kok, M. C.*, et al.* (2011). N-nitroso compound exposure-associated transcriptomic profiles are indicative of an increased risk for colorectal cancer. *Cancer Lett,* **309 :** 1-10.

Hebels, D. G., Sveje, K. M., de Kok, M. C.*, et al.* (2012). Red meat intake-induced increases in fecal water genotoxicity correlate with pro-carcinogenic gene expression changes in the human colon. *Food Chem Toxicol,* **50 :** 95-103.

Hodgson, J. M., Ward, N. C., Burke, V.*, et al.* (2007). Increased lean red meat intake does not elevate markers of oxidative stress and inflammation in humans. *J Nutr,* **137 :** 363-367.

Holland, J. D., Klaus, A., Garratt, A. N.*, et al.* (2013). Wnt signaling in stem and cancer stem cells. *Curr Opin Cell Biol,* **25 :** 254-264.

Hubner, R. A., Muir, K. R., Liu, J. F.*, et al.* (2008). Ornithine decarboxylase G316A genotype is prognostic for colorectal adenoma recurrence and predicts efficacy of aspirin chemoprevention. *Clin Cancer Res,* **14 :** 2303-2309.

Hughes, R., Cross, A. J., Pollock, J. R.*, et al.* (2001). Dose-dependent effect of dietary meat on endogenous colonic N-nitrosation. *Carcinogenesis,* **22 :** 199-202.

Ignatenko, N. A., Gerner, E. W. and Besselsen, D. G. (2011). Defining the role of polyamines in colon carcinogenesis using mouse models. *J Carcinog,* **10 :** 10.

Ignatenko, N. A., Yerushalmi, H. F., Pandey, R.*, et al.* (2009). Gene expression analysis of HCT116 colon tumor-derived cells treated with the polyamine analog PG-11047. *Cancer Genomics Proteomics,* **6 :** 161-175.

Ijssennagger, N., Rijnierse, A., de Wit, N.*, et al.* (2012). Dietary haem stimulates epithelial cell turnover by downregulating feedback inhibitors of proliferation in murine colon. *Gut,* **61 :** 1041-1049.

Ijssennagger, N., Rijnierse, A., de Wit, N. J.*, et al.* (2013). Dietary heme induces acute oxidative stress, but delayed cytotoxicity and compensatory hyperproliferation in mouse colon. *Carcinogenesis,* **34 :** 1628-1635.

Iliopoulos, D., Hirsch, H. A., Wang, G.*, et al.* (2011). Inducible formation of breast cancer stem cells and their dynamic equilibrium with non-stem cancer cells via IL6 secretion. *Proc Natl Acad Sci U S A,* **108 :** 1397-1402.

Innis, S. M. and Jacobson, K. (2007). Dietary lipids in early development and intestinal inflammatory disease. *Nutr Rev,* **65 :** S188-193.

Iso, T., Kedes, L. and Hamamori, Y. (2003). HES and HERP families: multiple effectors of the Notch signaling pathway. *J Cell Physiol,* **194 :** 237-255.

Jemal, A., Bray, F., Center, M. M.*, et al.* (2011). Global cancer statistics. *CA Cancer J Clin,* **61,** 69-90.

Joosen, A. M., Kuhnle, G. G., Aspinall, S. M., *et al.* (2009). Effect of processed and red meat on endogenous nitrosation and DNA damage. *Carcinogenesis,* **30 :** 1402-1407.

Joosen, A. M., Lecommandeur, E., Kuhnle, G. G., *et al.* (2010). Effect of dietary meat and fish on endogenous nitrosation, inflammation and genotoxicity of faecal water. *Mutagenesis,* **25 :** 243-247.

Kane, M. F., Loda, M., Gaida, G. M., *et al.* (1997). Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. *Cancer Res,* **57 :** 808-811.

Kang, G. H. (2011). Four molecular subtypes of colorectal cancer and their precursor lesions. *Arch Pathol Lab Med,* **135 :** 698-703.

Kanner, J. (2007). Dietary advanced lipid oxidation endproducts are risk factors to human health. *Mol Nutr Food Res,* **51 :** 1094-1101.

Kanthan, R., Senger, J. L. and Kanthan, S. C. (2012). Molecular events in primary and metastatic colorectal carcinoma: a review. *Patholog Res Int,* **2012 :** 597497.

Kanwal, R. and Gupta, S. (2012). Epigenetic modifications in cancer. *Clin Genet,* **81 :** 303-311.

Kesse, E., Clavel-Chapelon, F. and Boutron-Ruault, M. C. (2006). Dietary patterns and risk of colorectal tumors: a cohort of French women of the National Education System (E3N). *Am J Epidemiol,* **164 :** 1085-1093.

Kikuno, N., Shiina, H., Urakami, S., *et al.* (2008). Genistein mediated histone acetylation and demethylation activates tumor suppressor genes in prostate cancer cells. *Int J Cancer,* **123 :** 552-560.

King-Batoon, A., Leszczynska, J. M. and Klein, C. B. (2008). Modulation of gene methylation by genistein or lycopene in breast cancer cells. *Environ Mol Mutagen,* **49 :** 36-45.

Knekt, P., Jarvinen, R., Dich, J.*, et al.* (1999). Risk of colorectal and other gastro-intestinal cancers after exposure to nitrate, nitrite and N-nitroso compounds: a follow-up study. *Int J Cancer,* **80 :** 852-856.

Kodach, L. L., Bleuming, S. A., Peppelenbosch, M. P.*, et al.* (2007). The effect of statins in colorectal cancer is mediated through the bone morphogenetic protein pathway. *Gastroenterology,* **133 :** 1272-1281.

Kodach, L. L., Jacobs, R. J., Voorneveld, P. W.*, et al.* (2011). Statins augment the chemosensitivity of colorectal cancer cells inducing epigenetic reprogramming and reducing colorectal cancer cell 'stemness' via the bone morphogenetic protein pathway. *Gut,* **60 :** 1544-1553.

Kohli, M., Yu, J., Seaman, C.*, et al.* (2004). SMAC/Diablo-dependent apoptosis induced by nonsteroidal antiinflammatory drugs (NSAIDs) in colon cancer cells. *Proc Natl Acad Sci U S A,* **101 :** 16897-16902.

Kuhnle, G. G., Story, G. W., Reda, T.*, et al.* (2007). Diet-induced endogenous formation of nitroso compounds in the GI tract. *Free Radic Biol Med,* **43 :** 1040-1047.

Larque, E., Sabater-Molina, M. and Zamora, S. (2007). Biological significance of dietary polyamines. *Nutrition,* **23 :** 87-95.

Larsson, S. C., Rafter, J., Holmberg, L.*, et al.* (2005). Red meat consumption and risk of cancers of the proximal colon, distal colon and rectum: the Swedish Mammography Cohort. *Int J Cancer,* **113 :** 829-834.

Larsson, S. C. and Wolk, A. (2006). Meat consumption and risk of colorectal cancer: a meta-analysis of prospective studies. *Int J Cancer,* **119 :** 2657-2664.

Leslie, A., Carey, F. A., Pratt, N. R.*, et al.* (2002). The colorectal adenoma-carcinoma sequence. *Br J Surg,* **89 :** 845-860.

Lewin, M. H., Bailey, N., Bandaletova, T.*, et al.* (2006). Red meat enhances the colonic formation of the DNA adduct O6-carboxymethyl guanine: implications for colorectal cancer risk. *Cancer Res,* **66 :** 1859-1865.

Ley, S. H., Sun, Q., Willett, W. C.*, et al.* (2013). Associations between red meat intake and biomarkers of inflammation and glucose metabolism in women. *Am J Clin Nutr,* **2013,** 27.

Li, N., Xi, Y., Tinsley, H. N.*, et al.* (2013). Sulindac selectively inhibits colon tumor cell growth by activating the cGMP/PKG pathway to suppress Wnt/beta-catenin signaling. *Mol Cancer Ther,* **12 :** 1848-1859.

Liao, X., Lochhead, P., Nishihara, R.*, et al.* (2012). Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. *N Engl J Med,* **367 :** 1596-1606.

Liu, Q., Li, J., Cheng, R.*, et al.* (2013). Nitrosative stress plays an important role in Wnt pathway activation in diabetic retinopathy. *Antioxid Redox Signal,* **18 :** 1141-1153.

Mao, C., Zhou, J., Yang, Z.*, et al.* (2012). KRAS, BRAF and PIK3CA mutations and the loss of PTEN expression in Chinese patients with colorectal cancer. *PLoS ONE,* **7 :** e36653.

Marini, K. D., Payne, B. J., Watkins, D. N.*, et al.* (2011). Mechanisms of Hedgehog signalling in cancer. *Growth Factors,* **29 :** 221-234.

Markowitz, S. D. and Bertagnolli, M. M. (2009). Molecular origins of cancer: Molecular basis of colorectal cancer. *N Engl J Med,* **361 :** 2449-2460.

Martinez, M. E., O'Brien, T. G., Fultz, K. E., *et al.* (2003). Pronounced reduction in adenoma recurrence associated with aspirin use and a polymorphism in the ornithine decarboxylase gene. *Proc Natl Acad Sci U S A,* **100 :** 7859-7864.

McCullough, M. L., Gapstur, S. M., Shah, R., *et al.* (2013). Association between red and processed meat intake and mortality among colorectal cancer survivors. *J Clin Oncol,* **31 :** 2773-2782.

Medema, J. P. and Vermeulen, L. (2011). Microenvironmental regulation of stem cells in intestinal homeostasis and cancer. *Nature,* **474 :** 318-326.

Meyerhardt, J. A., Niedzwiecki, D., Hollis, D., *et al.* (2007). Association of dietary patterns with cancer recurrence and survival in patients with stage III colon cancer. *JAMA,* **298 :** 754-764.

Meyskens, F. L., Jr., McLaren, C. E., Pelot, D., *et al.* (2008). Difluoromethylornithine plus sulindac for the prevention of sporadic colorectal adenomas: a randomized placebo-controlled, double-blind trial. *Cancer Prev Res (Phila),* **1 :** 32-38.

Miller, P. E., Lazarus, P., Lesko, S. M., *et al.* (2013). Meat-related compounds and colorectal cancer risk by anatomical subsite. *Nutr Cancer,* **65 :** 202-226.

Mirvish, S. S., Haorah, J., Zhou, L., *et al.* (2003). N-nitroso compounds in the gastrointestinal tract of rats and in the feces of mice with induced colitis or fed hot dogs or beef. *Carcinogenesis,* **24 :** 595-603.

Miyamoto, S. and Rosenberg, D. W. (2011). Role of Notch signaling in colon homeostasis and carcinogenesis. *Cancer Sci,* **102 :** 1938-1942.

Montonen, J., Boeing, H., Fritsche, A., *et al.* (2013). Consumption of red meat and whole-grain bread in relation to biomarkers of obesity, inflammation, glucose metabolism and oxidative stress. *Eur J Nutr,* **52 :** 337-345.

Moonen, H. J., Dommels, Y. E., van Zwam, M., *et al.* (2004). Effects of polyunsaturated fatty acids on prostaglandin synthesis and cyclooxygenase-mediated DNA adduct formation by heterocyclic aromatic amines in human adenocarcinoma colon cells. *Mol Carcinog,* **40 :** 180-188.

Muzny, D. M., Bainbridge, M. N., Chang, K., *et al.* (2012). Comprehensive molecular characterization of human colon and rectal cancer. *Nature,* **487 :** 330-337.

Myers, B. R., Sever, N., Chong, Y. C., *et al.* (2013). Hedgehog pathway modulation by multiple lipid binding sites on the smoothened effector of signal response. *Dev Cell,* **26 :** 346-357.

Nachtergaele, S., Mydock, L. K., Krishnan, K., *et al.* (2012). Oxysterols are allosteric activators of the oncoprotein Smoothened. *Nat Chem Biol,* **8 :** 211-220.

Nishihara, R., Lochhead, P., Kuchiba, A., *et al.* (2013). Aspirin use and risk of colorectal cancer according to BRAF mutation status. *JAMA,* **309 :** 2563-2571.

Norat, T., Bingham, S., Ferrari, P., *et al.* (2005). Meat, fish, and colorectal cancer risk: the European Prospective Investigation into cancer and nutrition. *J Natl Cancer Inst,* **97 :** 906-916.

Norat, T., Lukanova, A., Ferrari, P., *et al.* (2002). Meat consumption and colorectal cancer risk: dose-response meta-analysis of epidemiological studies. *Int J Cancer,* **98 :** 241-256.

Oniscu, A., James, R. M., Morris, R. G., *et al.* (2004). Expression of Sonic hedgehog pathway genes is altered in colonic neoplasia. *J Pathol,* **203 :** 909-917.

Ouellet, V., Weisnagel, S. J., Marois, J.*, et al.* (2008). Dietary cod protein reduces plasma C-reactive protein in insulin-resistant men and women. *J Nutr,* **138 :** 2386-2391.

Paz, E. A., Lafleur, B. and Gerner, E. W. (2013). Polyamines are oncometabolites that regulate the LIN28/let-7 pathway in colorectal cancer cells. *Mol Carcinog,* **2013 :** 22051.

Pegg, A. E. (2009). Mammalian polyamine metabolism and function. *IUBMB Life,* **61 :** 880-894.

Peltomaki, P. (2012). Mutations and epimutations in the origin of cancer. *Exp Cell Res,* **318 :** 299-310.

Phillips, D. H. (1999). Polycyclic aromatic hydrocarbons in the diet. *Mutat Res,* **443 :** 139-147.

Pierre, F., Freeman, A., Tache, S.*, et al.* (2004). Beef meat and blood sausage promote the formation of azoxymethane-induced mucin-depleted foci and aberrant crypt foci in rat colons. *J Nutr,* **134 :** 2711-2716.

Pierre, F., Peiro, G., Tache, S.*, et al.* (2006). New marker of colon cancer risk associated with heme intake: 1,4-dihydroxynonane mercapturic acid. *Cancer Epidemiol Biomarkers Prev,* **15 :** 2274-2279.

Pierre, F., Santarelli, R., Tache, S.*, et al.* (2008). Beef meat promotion of dimethylhydrazine-induced colorectal carcinogenesis biomarkers is suppressed by dietary calcium. *Br J Nutr,* **99 :** 1000-1006.

Pierre, F., Tache, S., Gueraud, F.*, et al.* (2007). Apc mutation induces resistance of colonic cells to lipoperoxide-triggered apoptosis induced by faecal water from haem-fed rats. *Carcinogenesis,* **28 :** 321-327.

Pierre, F., Tache, S., Petit, C. R., *et al.* (2003). Meat and cancer: haemoglobin and haemin in a low-calcium diet promote colorectal carcinogenesis at the aberrant crypt stage in rats. *Carcinogenesis,* **24 :** 1683-1690.

Pierre, F. H., Santarelli, R. L., Allam, O., *et al.* (2010). Freeze-dried ham promotes azoxymethane-induced mucin-depleted foci and aberrant crypt foci in rat colon. *Nutr Cancer,* **62 :** 567-573.

Polakis, P. (2012). Wnt signaling in cancer. *Cold Spring Harb Perspect Biol,* **4 :** a008052.

Portela, A. and Esteller, M. (2010). Epigenetic modifications and human disease. *Nat Biotechnol,* **28 :** 1057-1068.

Pot, G. K., Geelen, A., Majsak-Newman, G., *et al.* (2010). Increased consumption of fatty and lean fish reduces serum C-reactive protein concentrations but not inflammation markers in feces and in colonic biopsies. *J Nutr,* **140 :** 371-376.

Pot, G. K., Geelen, A., van Heijningen, E. M., *et al.* (2008). Opposing associations of serum n-3 and n-6 polyunsaturated fatty acids with colorectal adenoma risk: an endoscopy-based case-control study. *Int J Cancer,* **123 :** 1974-1977.

Puglisi, M. A., Tesori, V., Lattanzi, W., *et al.* (2013). Colon cancer stem cells: Controversies and perspectives. *World J Gastroenterol,* **19 :** 2997-3006.

Puppa, G., Sonzogni, A., Colombari, R., *et al.* (2010). TNM staging system of colorectal carcinoma: a critical appraisal of challenging issues. *Arch Pathol Lab Med,* **134 :** 837-852.

Qiu, W., Wang, X., Leibowitz, B., *et al.* (2010). Chemoprevention by nonsteroidal anti-inflammatory drugs eliminates oncogenic intestinal stem cells via SMAC-dependent apoptosis. *Proc Natl Acad Sci U S A,* **107 :** 20027-20032.

Radtke, F., Clevers, H. and Riccio, O. (2006). From gut homeostasis to cancer. *Curr Mol Med,* **6 :** 275-289.

Raj, K. P., Zell, J. A., Rock, C. L.*, et al.* (2013). Role of dietary polyamines in a phase III clinical trial of difluoromethylornithine (DFMO) and sulindac for prevention of sporadic colorectal adenomas. *Br J Cancer,* **108 :** 512-518.

Reddy, B. S. (2004). Omega-3 fatty acids in colorectal cancer prevention. *Int J Cancer,* **112 :** 1-7.

Reedijk, M., Odorcic, S., Zhang, H.*, et al.* (2008). Activation of Notch signaling in human colon adenocarcinoma. *Int J Oncol,* **33 :** 1223-1229.

Renehan, A. G., Roberts, D. L. and Dive, C. (2008). Obesity and cancer: pathophysiological and biological mechanisms. *Arch Physiol Biochem,* **114 :** 71-83.

Riobo, N. A. (2012). Cholesterol and its derivatives in Sonic Hedgehog signaling and cancer. *Curr Opin Pharmacol,* **12 :** 736-741.

Rodriguez, I. R. and Larrayoz, I. M. (2010). Cholesterol oxidation in the retina: implications of 7KCh formation in chronic inflammation and age-related macular degeneration. *J Lipid Res,* **51 :** 2847-2862.

Ronchini, C. and Capobianco, A. J. (2001). Induction of cyclin D1 transcription and CDK2 activity by Notch(ic): implication for cell cycle disruption in transformation by Notch(ic). *Mol Cell Biol,* **21 :** 5925-5934.

Rustgi, A. K. (2007). The genetics of hereditary colon cancer. *Genes Dev,* **21 :** 2525-2538.

Sadanandam, A., Wang, X., de Sousa, E. M. F., *et al.* (2014). Reconciliation of classification systems defining molecular subtypes of colorectal cancer: interrelationships and clinical implications. *Cell Cycle,* **13 :** 353-357.

Samuels, Y., Wang, Z., Bardelli, A., *et al.* (2004). High frequency of mutations of the PIK3CA gene in human cancers. *Science,* **304 :** 554.

Sandhu, M. S., White, I. R. and McPherson, K. (2001). Systematic review of the prospective cohort studies on meat consumption and colorectal cancer risk: a meta-analytical approach. *Cancer Epidemiol Biomarkers Prev,* **10 :** 439-446.

Sandler, R. S., Halabi, S., Baron, J. A., *et al.* (2003). A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. *N Engl J Med,* **348 :** 883-890.

Santarelli, R. L., Pierre, F. and Corpet, D. E. (2008). Processed meat and colorectal cancer: a review of epidemiologic and experimental evidence. *Nutr Cancer,* **60 :** 131-144.

Santarelli, R. L., Vendeuvre, J. L., Naud, N., *et al.* (2010). Meat processing and colon carcinogenesis: cooked, nitrite-treated, and oxidized high-heme cured meat promotes mucin-depleted foci in rats. *Cancer Prev Res (Phila),* **3 :** 852-864.

Scholer-Dahirel, A., Schlabach, M. R., Loo, A., *et al.* (2011). Maintenance of adenomatous polyposis coli (APC)-mutant colorectal cancer is dependent on Wnt/beta-catenin signaling. *Proc Natl Acad Sci U S A,* **108 :** 17135-17140.

Schwitalla, S., Fingerle, A. A., Cammareri, P., *et al.* (2013). Intestinal Tumorigenesis Initiated by Dedifferentiation and Acquisition of Stem-Cell-like Properties. *Cell,* **152 :** 25-38.

Segditsas, S. and Tomlinson, I. (2006). Colorectal cancer and genetic alterations in the Wnt pathway. *Oncogene,* **25 :** 7531-7537.

Sesink, A. L., Termont, D. S., Kleibeuker, J. H., *et al.* (1999). Red meat and colon cancer: the cytotoxic and hyperproliferative effects of dietary heme. *Cancer Res,* **59 :** 5704-5709.

Sesink, A. L., Termont, D. S., Kleibeuker, J. H., *et al.* (2000). Red meat and colon cancer: dietary haem, but not fat, has cytotoxic and hyperproliferative effects on rat colonic epithelium. *Carcinogenesis,* **21 :** 1909-1915.

Shaker, A. and Rubin, D. C. (2010). Intestinal stem cells and epithelial-mesenchymal interactions in the crypt and stem cell niche. *Transl Res,* **156 :** 180-187.

Sharma, S., Kelly, T. K. and Jones, P. A. (2010). Epigenetics in cancer. *Carcinogenesis,* **31 :** 27-36.

Sikandar, S. S., Pate, K. T., Anderson, S., *et al.* (2010). NOTCH signaling is required for formation and self-renewal of tumor-initiating cells and for repression of secretory cell differentiation in colon cancer. *Cancer Res,* **70 :** 1469-1478.

Simopoulos, A. P. (2006). Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases. *Biomed Pharmacother,* **60 :** 502-507.

Sinha, R., Peters, U., Cross, A. J., *et al.* (2005). Meat, meat cooking methods and preservation, and risk for colorectal adenoma. *Cancer Res,* **65 :** 8034-8041.

Skinner, M. K., Manikkam, M. and Guerrero-Bosagna, C. (2010). Epigenetic transgenerational actions of environmental factors in disease etiology. *Trends Endocrinol Metab,* **21 :** 214-222.

Slattery, M. L., Boucher, K. M., Caan, B. J., *et al.* (1998). Eating patterns and risk of colon cancer. *Am J Epidemiol,* **148 :** 4-16.

Snover, D. C. (2011). Update on the serrated pathway to colorectal carcinoma. *Hum Pathol,* **42 :** 1-10.

Soerjomataram, I., Lortet-Tieulent, J., Parkin, D. M.*, et al.* (2012). Global burden of cancer in 2008: a systematic analysis of disability-adjusted life-years in 12 world regions. *Lancet,* **380 :** 1840-1850.

Spann, N. J. and Glass, C. K. (2013). Sterols and oxysterols in immune cell function. *Nat Immunol,* **14 :** 893-900.

Stavric, B. (1994). Biological significance of trace levels of mutagenic heterocyclic aromatic amines in human diet: a critical review. *Food Chem Toxicol,* **32 :** 977-994.

Sturla, S. J., Boobis, A. R., Fitzgerald, R. E.*, et al.* (2014). Systems Toxicology: From Basic Research to Risk Assessment. *Chem Res Toxicol,* **27 :** 314-329.

Sugimura, T., Wakabayashi, K., Nakagama, H.*, et al.* (2004). Heterocyclic amines: Mutagens/carcinogens produced during cooking of meat and fish. *Cancer Sci,* **95 :** 290-299.

Sun, K., Deng, H. J., Lei, S. T.*, et al.* (2013). miRNA-338-3p suppresses cell growth of human colorectal carcinoma by targeting smoothened. *World J Gastroenterol,* **19 :** 2197-2207.

Suzuki, H., Maruyama, R., Yamamoto, E.*, et al.* (2013). Epigenetic alteration and microRNA dysregulation in cancer. *Front Genet,* **4 :** 258.

Takebe, N., Harris, P. J., Warren, R. Q.*, et al.* (2011). Targeting cancer stem cells by inhibiting Wnt, Notch, and Hedgehog pathways. *Nat Rev Clin Oncol,* **8 :** 97-106.

Tanaka, J., Watanabe, T., Kanazawa, T.*, et al.* (2007). Left-Sided microsatellite unstable colorectal cancers show less frequent methylation of hMLH1 and CpG island methylator phenotype than right-sided ones. *J Surg Oncol,* **96 :** 611-618.

Thomson, T. M., Sewer, A., Martin, F.*, et al.* (2013). Quantitative assessment of biological impact using transcriptomic data and mechanistic network models. *Toxicol Appl Pharmacol,* **272 :** 863-878.

Tinsley, H. N., Gary, B. D., Thaiparambil, J.*, et al.* (2010). Colon tumor cell growth-inhibitory activity of sulindac sulfide and other nonsteroidal anti-inflammatory drugs is associated with phosphodiesterase 5 inhibition. *Cancer Prev Res (Phila),* **3 :** 1303-1313.

Toyota, M., Ahuja, N., Ohe-Toyota, M.*, et al.* (1999). CpG island methylator phenotype in colorectal cancer. *Proc Natl Acad Sci U S A,* **96 :** 8681-8686.

Tricker, A. R. and Preussmann, R. (1991). Carcinogenic N-nitrosamines in the diet: occurrence, formation, mechanisms and carcinogenic potential. *Mutat Res,* **259 :** 277-289.

Trifan, O. C. and Hla, T. (2003). Cyclooxygenase-2 modulates cellular growth and promotes tumorigenesis. *J Cell Mol Med,* **7 :** 207-222.

Tsai, H. C. and Baylin, S. B. (2011). Cancer epigenetics: linking basic biology to clinical medicine. *Cell Res,* **21 :** 502-517.

Ullman, T. A. and Itzkowitz, S. H. (2011). Intestinal inflammation and cancer. *Gastroenterology,* **140 :** 1807-1816.

Uno, S., Endo, K., Jeong, Y.*, et al.* (2009). Suppression of beta-catenin signaling by liver X receptor ligands. *Biochem Pharmacol,* **77 :** 186-195.

Ushijima, T. (2005). Detection and interpretation of altered methylation patterns in cancer cells. *Nat Rev Cancer,* **5 :** 223-231.

Waldner, M. J., Foersch, S. and Neurath, M. F. (2012). Interleukin-6--a key regulator of colorectal cancer development. *Int J Biol Sci,* **8 :** 1248-1253.

van der Flier, L. G. and Clevers, H. (2009). Stem cells, self-renewal, and differentiation in the intestinal epithelium. *Annu Rev Physiol,* **71 :** 241-260.

van der Meer-van Kraaij, C., Kramer, E., Jonker-Termont, D.*, et al.* (2005). Differential gene expression in rat colon by dietary heme and calcium. *Carcinogenesis,* **26 :** 73-79.

van Duijnhoven, F. J., Bueno-De-Mesquita, H. B., Ferrari, P.*, et al.* (2009). Fruit, vegetables, and colorectal cancer risk: the European Prospective Investigation into Cancer and Nutrition. *Am J Clin Nutr,* **89 :** 1441-1452.

van Es, J. H., van Gijn, M. E., Riccio, O.*, et al.* (2005). Notch/gamma-secretase inhibition turns proliferative cells in intestinal crypts and adenomas into goblet cells. *Nature,* **435 :** 959-963.

van Loon, A. J., Botterweck, A. A., Goldbohm, R. A.*, et al.* (1998). Intake of nitrate and nitrite and the risk of gastric cancer: a prospective cohort study. *Br J Cancer,* **78 :** 129-135.

van Woudenbergh, G. J., Kuijsten, A., Tigcheler, B.*, et al.* (2012). Meat consumption and its association with C-reactive protein and incident type 2 diabetes: the Rotterdam Study. *Diabetes Care,* **35 :** 1499-1505.

Wang, X., Lei, X. G. and Wang, J. (2014). Malondialdehyde regulates glucose-stimulated insulin secretion in murine islets via TCF7L2-dependent Wnt signaling pathway. *Mol Cell Endocrinol,* **382 :** 8-16.

Wang, X., Liu, X., Li, A. Y.*, et al.* (2011). Overexpression of HMGA2 promotes metastasis and impacts survival of colorectal cancers. *Clin Cancer Res,* **17 :** 2570-2580.

Vargas, A. J., Wertheim, B. C., Gerner, E. W.*, et al.* (2012). Dietary polyamine intake and risk of colorectal adenomatous polyps. *Am J Clin Nutr,* **96 :** 133-141.

Varnat, F., Duquet, A., Malerba, M., *et al.* (2009). Human colon cancer epithelial cells harbour active HEDGEHOG-GLI signalling that is essential for tumour growth, recurrence, metastasis and stem cell survival and expansion. *EMBO Mol Med,* **1 :** 338-351.

WCRF/AICR. (2007). Nutrition, physical activity and the prevention of cancer: a global perspective., World Cancer Research Fund/American Institute for Cancer Research Food, Washington DC.

Weisenberger, D. J., Siegmund, K. D., Campan, M., *et al.* (2006). CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. *Nat Genet,* **38 :** 787-793.

Vejux, A. and Lizard, G. (2009). Cytotoxic effects of oxysterols associated with human diseases: Induction of cell death (apoptosis and/or oncosis), oxidative and inflammatory activities, and phospholipidosis. *Mol Aspects Med,* **30 :** 153-170.

Wend, P., Holland, J. D., Ziebold, U., *et al.* (2010). Wnt signaling in stem and cancer stem cells. *Semin Cell Dev Biol,* **21 :** 855-863.

Weng, A. P., Millholland, J. M., Yashiro-Ohtani, Y., *et al.* (2006). c-Myc is an important direct target of Notch1 in T-cell acute lymphoblastic leukemia/lymphoma. *Genes Dev,* **20 :** 2096-2109.

Vermeulen, L., De Sousa, E. M. F., van der Heijden, M., *et al.* (2010). Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nat Cell Biol,* **12 :** 468-476.

White, B. D., Chien, A. J. and Dawson, D. W. (2012). Dysregulation of Wnt/beta-catenin signaling in gastrointestinal cancers. *Gastroenterology,* **142 :** 219-232.

Wilson, B. J., Schatton, T., Frank, M. H.*, et al.* (2011). Colorectal Cancer Stem Cells: Biology and Therapeutic Implications. *Curr Colorectal Cancer Rep,* **7 :** 128-135.

Wirfalt, E., Midthune, D., Reedy, J.*, et al.* (2009). Associations between food patterns defined by cluster analysis and colorectal cancer incidence in the NIH-AARP diet and health study. *Eur J Clin Nutr,* **63 :** 707-717.

Visvader, J. E. (2011). Cells of origin in cancer. *Nature,* **469 :** 314-322.

Visvader, J. E. and Lindeman, G. J. (2012). Cancer stem cells: current status and evolving complexities. *Cell Stem Cell,* **10 :** 717-728.

Vogt, T., McClelland, M., Jung, B.*, et al.* (2001). Progression and NSAID-induced apoptosis in malignant melanomas are independent of cyclooxygenase II. *Melanoma Res,* **11 :** 587-599.

Wood, L. D., Parsons, D. W., Jones, S.*, et al.* (2007). The genomic landscapes of human breast and colorectal cancers. *Science,* **318 :** 1108-1113.

Xu, W., Tamim, H., Shapiro, S.*, et al.* (2006). Use of antidepressants and risk of colorectal cancer: a nested case-control study. *Lancet Oncol,* **7 :** 301-308.

Yoon, J. H., Canbay, A. E., Werneburg, N. W.*, et al.* (2004). Oxysterols induce cyclooxygenase-2 expression in cholangiocytes: implications for biliary tract carcinogenesis. *Hepatology,* **39 :** 732-738.

Yu, H., Pardoll, D. and Jove, R. (2009). STATs in cancer inflammation and immunity: a leading role for STAT3. *Nat Rev Cancer,* **9 :** 798-809.

Zell, J. A., Ignatenko, N. A., Yerushalmi, H. F.*, et al.* (2007). Risk and risk reduction involving arginine intake and meat consumption in colorectal tumorigenesis and survival. *Int J Cancer,* **120 :** 459-468.

Zell, J. A., Lin, B. S., Ziogas, A.*, et al.* (2012). Meat consumption, ornithine decarboxylase gene polymorphism, and outcomes after colorectal cancer diagnosis. *J Carcinog,* **11 :** 17.

Zhu, S., Wu, H., Wu, F.*, et al.* (2008). MicroRNA-21 targets tumor suppressor genes in invasion and metastasis. *Cell Res,* **18 :** 350-359.

FIGURE LEGENDS

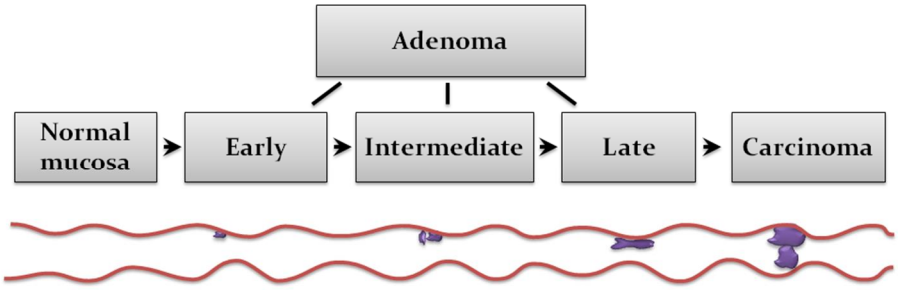

Figure 1

Simplified representation of multistage colorectal carcinogenesis, based on a model originally proposed by Fearon and Vogelsterin, 1990. Mutations to either of *APC* or *CTNNB1* (the ß-catenin gene) of the Wnt signaling cascade occur in 80 % - 90 % of sporadic adenocarcinomas. Such genetic changes are perceived as initial events and can alone generate pre-neoplastic lesions. Activation of KRAS, reported in about 40 % of CRCs, is thought to promote development of colorectal adenomas. Inactivating somatic mutations of *TGFBRII* (encoding a receptor of TGF-ƀ) is observed in about 33 % of CRCs and believed to promote transition of adenoma to advanced dysplasia. Subsequent stages, contributing to downstream cancer progression, may include mutational activation of *PIK3CA*, encoding an essential component of the PI3K signal transduction pathway, inactivation of *DCC* and *TP53* ï key tumor suppressor

genes - as well as *SMAD4*, a core entity of the BMP pathway. Even later steps, linked with advanced and metastatic disease, can involve inactivation of *PTEN*, likewise a suppressor gene. Although this straightforward scheme may roughly apply to many instances of sporadic colorectal carcinogenesis, the current perception is more elaborated and involves ï apart from genomic mutations touched upon above - several sub-categories of genomic instability, microRNA and epigenetic changes. Moreover, besides colorectal tumor development from adenomatous lesions, recent findings suggest that a similar sequence of events to overt malignancy can likewise occur from serrated polyps; a route referred to as the Serrated Neoplastic Pathway. Lastly, certain hereditary CRC varieties, such as Juvenile Polyposis, can involve quite distinct molecular changes and thus routes to malignancy not typically seen in sporadic cases.

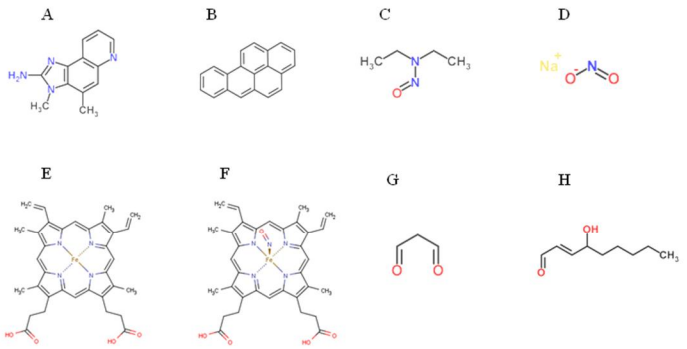

Figure 2

Molecular structures of key bioactive chemicals referred to in the text. **A**: 2-amino-3,4-dimethylimidazo[4,5]quinoline; an example mutagenic heterocyclic amine which appears on meat cooked at high temperature. **B**: Benzo[a]pyrene; a representative carcinogenic polyaromatic hydrocarbon (PAH) present on smoked, barbequed and high-temperature fried meat. **C**: Diethylnitrosamine; a typical N-nitrosamine which can appear intestinally as a consequence of N-nitrosation of peptide-derived amines or amides. **D**: Sodium nitrite; a common food additive used to preserve or cure meat. **E**: Haem (ferroprotoporphyrin IX); abundant in myoglobulin of red meat. **F**: Nitrosyl-haem; formed in nitrite-cured red meat and being attributed to higher intestinal carcinogenicity than haem. **G**: Malondialdehyde; an oxidation product of polyunsaturated fatty acids. **H**: 4-hydroxynonenal; a major oxidation aldehyde product of membrane lipids containing n-6 polyunsaturated fatty acids.

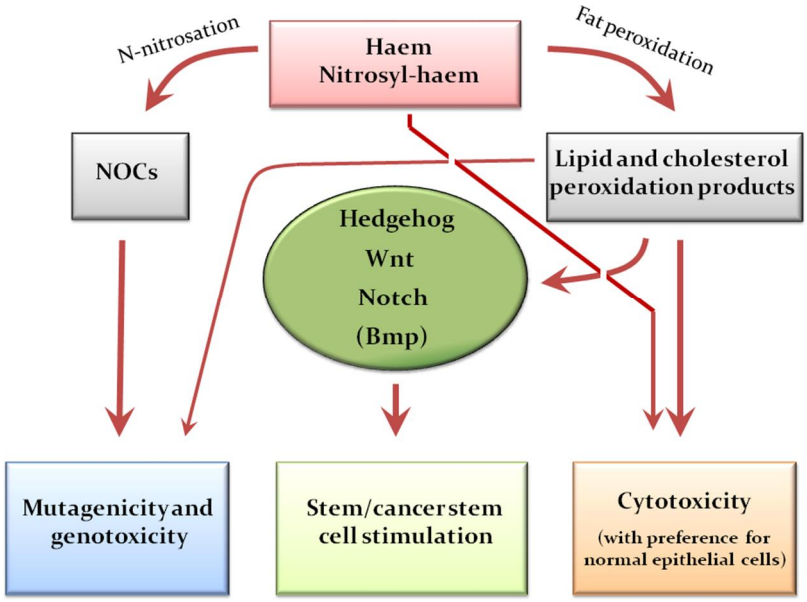

Figure 3

Major components as well as several known and tentative reactions and interaction schemes in red/processed meat-related development of colorectal carcinoma. Haem and nitrosyl-haem, the latter present in nitrite cured processed meat, catalyze a series of N-nitrosation and fat peroxidation reactions, leading to the formation of various N-nitroso compounds such as diethylnitrosamine (see Fig 2) and fat peroxidation products, typified by malondialdehyde and 4-HNE (see Fig 2). The former class of compounds are known to produce DNA adducts and mutations, whereas the latter category, collectively, has broad effect range involving all of those specified in boxes at the bottom part of the figure. For instance, 4-HNE is known to selectively induce cytotoxicity in normal intestinal epithelial cells, whereas premalignant counterparts are largely protected. Further, certain oxidation products of lipids and cholesterol are reported to interact with developmental genes central to cellular migration and differentiation. Notably, 4-HNE can trigger the expression of both Hedgehog and Wnt, as shown in a non-epithelial

experimental system. Malondialdehyde is likewise connected with Wnt activation. Certain oxysterols can activate Smo of the Hedgehog signaling cascade, potentially leading to downstream Wnt stimulation. The depicted route from lipid oxidation products - via developmental signal transduction cascades - to the promotion of (normal or cancer) epithelial stem cells is tentative, but indirectly supported by many observations. Lastly, recent observations suggest that haem (or a lipid derivative) can itself induce intestinal cytotoxicity, which - in turn - triggers epithelial hyper-proliferation.