8

Lipid Peroxidation in Colorectal Carcinogenesis: Bad and Good News

Stefania Pizzimenti, Cristina Toaldo,
Piergiorgio Pettazzoni, Eric Ciamporcero,
Mario Umberto Dianzani and Giuseppina Barrera
University of Turin,
Italy

1. Introduction

During oxidative stress, membrane lipids are one of the major targets of Reactive Oxygen Species (ROS) that are known to elicit oxidative decomposition of polyunsaturated fatty acids (PUFAs) of membrane phospholipids, a process usually referred to lipid peroxidation (Esterbauer et al., 1991). During this process, a number of carbonylic compounds are generated as final products, including acrolein, malondialdehyde (MDA) and 4-hydroxyalkenals (Esterbauer et al., 1991). Among the 4-hydroxyalkenal class, 4-hydroxynonenal (HNE) is the most abundant aldehyde produced (Dianzani et al., 1999). Over the years, HNE has achieved a status as one of the best recognized and most studied of the cytotoxic products of lipid peroxidation (Poli et al., 2008). In addition to studies on its bioactivity, HNE is commonly used as a biomarker for the occurrence and/or the extent of oxidative stress. It appears to be produced specifically by peroxidation of ω-6 PUFAs, such as linoleic acid, arachidonic acid (AA) and γ-linolenic acid (Esterbauer et al., 1982). HNE has three main functional groups: the aldehyde group, the C=C double bond and the hydroxyl group, which can participate, alone or in sequence, in chemical reactions with other molecules (Esterbauer et al., 1991). HNE is a highly electrophilic molecule, which predisposes it to localize in the cell membranes. It can easily react with low molecular weight compounds, such as glutathione, with proteins, with lipids and, at higher concentration, with DNA (Esterbauer et al., 1991; Uchida, 2003). The double bond, the carbonyl group and the hydroxyl group, all contribute to making HNE highly reactive with nucleophiles with the primary reactivity of the molecule lying at the unsaturated bond of the C-3 atom. HNE has been shown to form Michael adducts via the C-3 atom with the sulfhydryl group of Cys residues, the imidazole group of His residues, and the ε-amino group of Lys residues on a large number of proteins (Esterbauer et al., 1991). Recently, it has been proposed that HNE can also modify Arg residues of proteins (Isom et al., 2004). In addition to Michael adduct formation, Lys residues also form Schiff bases and pentylpyrrole adducts with HNE via the C-1 aldehyde group (Sayre et al., 1993; Petersen & Doorn, 2004; Schaur, 2003)

HNE-modified proteins can be removed by the proteasomal system (Siems & Grune, 2003).

Once formed, HNE is rapidly degraded and its metabolism is dependent upon a set of specific enzymes presenting high affinity toward HNE. In particular HNE metabolism can be divided into glutathione-mediated and oxidative/reductive categories (Siems & Grune, 2003). In the first case, HNE binds the thiol group of GSH resulting in the correspondent emiacetal (Balogh & Atkins, 2011). The reaction with GSH can occur in a spontaneous manner, with low efficiency or through the reaction catalized by Glutathione S Transferases (GSTs). Moreover, although a number of isoforms of GST manifested HNE conjugating activity, it has been widely reported that the isoform GST A4 presents the highest HNE affinity (Balogh & Atkins , 2011). The oxidative/reductive pathway of HNE involves its NAD/NADP-dependent oxidative conversion to 4-hydroxy-2-nonenoic Acid (HNA) catalyzed by aldheyde dehydrogenases (ALDHs) or the reductive conversion to 1,4-dihydroxy-2-nonene (DHN) catalyzed by alchool dehydrogenase (ADH) or aldhehyde reductase (AR) (Hartley et al., 1995; Vander Jagt et al., 1995). However, the majority of HNE is metabolized through forming GS-HNE (Forman et al., 2003). HNE-GSH is then further metabolized and found in urine, mostly, as the mercapturic acid derivative, HNE-MA (Alary, 1995). Indeed, HNE-GSH adduct is further metabolized by γ-glutamyltranspeptidase (γ-GT) and dipeptidases (DP) to the cysteinyl (CYS)-HNE thioether adduct. The cysteinyl thioether adduct is a substrate for acetyltransferases (AT) that catalyze the acetylation of the cysteinyl adduct to generate the acetylcysteinyl (AcCYS), or mercapturic acid, adduct. HNE metabolites also can be found associated with mercapturic acid, such as DHN-MA, HNE-MA and HNA-lactone (Alary et al., 2003). HNE is also partially excreted first with the bile, then with the faeces, under the form of conjugated metabolites. However, biliary metabolites undergo an enterohepatic cycle that limits the final excretion of faecal metabolites (Alary et al., 2003).

HNE, and in general aldehydes formed during membrane lipid peroxidation, are quite long lived, as compared to reactive free radicals and can widely diffuse and react around the site of origin (Esterbauer 1991). As a consequence, HNE and related aldehydes were proposed as putative ultimate toxic messengers, potentially able to mediate stress-related injury at the molecular level (Uchida, 2003). Indeed, HNE has been detected in vivo in several pathological conditions, which entail increased lipid peroxidation, including inflammation, atherosclerosis, chronic degenerative diseases of the nervous system, and chronic liver diseases, reaching a concentration up to about 10 μM (Parola et al., 1999).

However, under physiological conditions, HNE can be found at low concentrations in human tissues and plasma (0.07-2.8 μM) (Esterbauer et al., 1991, Poli et al., 2008) where it participates in the control of biological processes, such as signal transduction, cell proliferation and differentiation. Indeed, HNE, similarly to ROS, plays an important role in controlling the intracellular signal transduction pathways involved in a number of cell responses (Parola et al., 1999; Dianzani et al., 2003; Leonarduzzi et al., 2004).

The contribution of HNE and lipid peroxidation in carcinogenesis is still controversial. Beside pro-tumoral effects, several authors pointed out their protective role. This "two-faced" role has already emerged for ROS (Halliwell, 2007; Wang & Yi, 2008; Pan et al., 2009; Acharya et al., 2010) and increasing evidence is emerging also for a dual role of lipid peroxidation products (Zhi-Hua et al., 2006; Pizzimenti et al., 2010a).

2. HNE and carcinogenesis

2.1 DNA-adducts, mutagenicity and genotoxicity

2.1.1 DNA-adducts

The most substantial evidence of the genotoxic and mutagenic effect of HNE is the formation of HNE-DNA adducts. ROS and HNE seem to share this feature and this has been proposed as the mechanism of tumor induction (Bartsch & Nair, 2005).

One of the most studied HNE-adducts is the propane-type DNA adduct with deoxyguanosine, the 6-(1-hydroxyhexanyl)-8-hydroxy-1, N2-propano-2'deoxyguanine (HNE-dG) (Winter et al., 1986). AA appears to be a major source of HNE-DNA adducts, producing a total of 20.6 µmol of HNE-dG adducts (Chung et al., 2000), by an in vitro assay using 1 mM AA. HNE-dG is also the main lesion produced upon the addition of HNE to DNA (Chung et al., 2000; Wacker et al., 2001); moreover, an increase of HNE-dG adducts was observed in the liver DNA of rats after treatment with CCl4, a well known inducer of lipid peroxidation (Chung et al., 2000). Taken together, these results generate substantial evidence for the endogenous formation of these adducts, thus it has been proposed that lipid peroxidation is a main endogenous pathway leading to propano adduction in DNA (Chung et al., 1999).

In the presence of peroxides and reactive oxygen species, HNE can be further metabolized to an epoxide intermediate that interacts with DNA, forming etheno-type DNA adducts (Chung et al., 1996). However the etheno-type DNA adducts are produced in significantly lower yield, with respect to the HNE-dG adducts, when a pro-oxidant stimulus, such H_2O_2, or HNE is added to cells. Indeed, in these experimental conditions, HNE-dG represent more than 95% of the overall adducts to DNA, suggesting that HNE-dG may represent the best biomarker of the genotoxic effects of HNE (Douki et al., 2004).

All four bases of DNA are the targets for HNE adduct formation (Chung et al., 1996; De Bont et al., 2004), but with different efficiency: G>C>A>T (Kowalczyk et al., 2004).

HNE-DNA adducts have been identified in tissues of untreated rats and humans (Chung et al., 2000), suggesting that the endogenously produced HNE can form adducts with DNA in the physiological condition also.

Removal of these modified bases from DNA plays an important role in the prevention of mutagenesis and carcinogenesis. Each cell has an efficient defence mechanism to repair these types of damage via DNA repair pathways such as base excision repair (BER), nucleotide excision repair (NER), and mismatch repair (MMR) pathways (Min & Eberel, 2009). In human leukocyte treated with 200 µM HNE, DNA damage was repaired after 12 h and returned to the control level at 24 h (Park & Park, 2011).

It has been demonstrated that NER is a major pathway for repairing HNE-dG adducts, since HNE-dG adducts induce a significantly higher level of genotoxicity and mutagenicity in NER-deficient human and E. coli cells than in NER-proficient cells (Feng et al., 2003). Moreover, other authors suggested that HNE can also contribute to carcinogenesis, by inhibiting the nucleotide excision repair (NER) of DNA damage in cancer cells with concentration higher than 50 µM (Feng et al., 2004). In any case, HNE forms adducts with DNA only at higher concentrations, since it can react quickly with amino and sulphydrylic groups of proteins and, primarly, with the sulphydrylic group of GSH. Indeed, it has been calculated that GSH conjugates were 20000 times more numerous than DNA adducts when HNE was exogenously added to the cultured cells (Falletti & Douki, 2008).

2.1.2 Mutagenicity

In this context, it would be important to know whether the HNE-dG adducts are mutagenic. Several authors suggest this possibility, since HNE has been shown to be mutagenic in mammalian cells (Cajelli et al., 1987). HNE was negative in bacterial mutagenicity tests, however its epoxidized form has been tested positive (Chung et al., 1993). HNE was found to be responsible for recombination, base substitutions and frameshift mutations in M13 phage transfected in E.coli (Kowalczyk et al., 2004).

Moreover it has been reported that 50 μM HNE treatment in human cells induces a high frequency of G.C to T.A mutations at the third base of codon 249 (AGG*) of the p53 gene (Hussain et al., 2000), a mutational hot spot in human cancers, particularly in hepatocellular carcinoma (Hsu et al., 1991). Both eheno and propane type HNE-DNA-adduct at codon 249 can be responsible for such transitions (Feng et al., 2003).

The stereochemistry of HNE-dG adducts seems to play an important role in determining mutations. Indeed, two of the HNE-dG adducts, (6R, 8S, 11R) and (6S, 8R, 11S), were significantly more mutagenic than (6R, 8S, 11S) and (6S, 8R, 11R) HNE-dG adducts. Only one of the HNE stereoisomers was able to form interstrand DNA–DNA cross-links. (Fernandes et al., 2003).

2.1.3 Genotoxicity

The genotoxic property of HNE was demonstrated in different cell types, such as on cultured human lymphocytes (Emerit et al., 1991), in primary hepatocytes (Esterbauer et al., 1991) and cerebral microvascular endothelial cells (Eckl, 2003). In these cell lines an increase of micronuclei (a biomarker of chromosome breakage and/or whole chromosome loss), chromosomal aberrations and sister chromatid exchanges was observed after exposure to HNE at relatively low doses, ranging 0.1-10 μM. However these clastrogenic features of low doses of HNE failed to be confirmed in a recent multicentrum study on DNA of normal peripheral blood lymphocytes (Katic et al., 2010).

Currently, the comet assay has been extensively used to measure DNA strand breaks, since it represents a sensitive and rapid assay to detect the mutagenic and genotoxicity of chemicals and xenobiotics (Tice, et al., 1991).

Unfortunately, most HNE-induced DNA lesions are the stable 1,N2-propano adducts and they are not detected by this technique. By using this assay, the genotoxic property of 5-10 μM HNE in the K562 leukemic cell line has been shown; this feature was highly dependent on cellular GSH/GST/AR system (Yadav et al., 2008). The comet test was also used to demonstrate the genotoxicity of 200 μM HNE in human leukocytes (Park & Park, 2011).

2.2 Results in laboratory animals

To date, in contrast with several in vitro experimental results, tumor bioassays in laboratory animals failed to demonstrate the carcinogenic and mutagenic properties of HNE. HNE, in particular its epoxy derivate, has shown that to be a weak tumor-initiating agent, causing the development of renal preneoplastic tubule lesions in new-born mice (Chung et al., 1993). More interestingly, HNE lacks in vivo genotoxicity in lacI transgenic mice, a model for detecting mutagenicity in target organs, even when lethal doses are applied (Nishikawa et al., 2000).

The big gap between the in vitro and the in vivo data can be partially explained by carefully considering the elevated doses frequently used to demonstrated the carcinogenic properties

of HNE in vitro. Indeed, several mutagenic assays with HNE have been performed with high doses of HNE (more than 100 μM). It seems rather unlikely that HNE or other aldehydes can reach overall concentrations in the range of 100 μM in cells and organs (Esterbauer et al., 1991). It is conceivable that such levels may be built up locally, near or within peroxidizing membranes for a short time because of their high lipophilicity. It has been calculated, for example, that the concentration of HNE in the lipid bilayer of isolated peroxidizing microsomes is about 4.5 mM (Koster et al., 1986). Nevertheless, a convincing demonstration that this very high concentration can be reached into the cells has remained elusive. On the other hand, when HNE diffuses out from membranes, its concentration is reduced by the surrounding aqueous phase. Moreover, the cytosolic HNE-metabolizing enzymes destroy HNE produced in excess so that the steady-state HNE concentration into the cells, around 1 μM, is reached quickly (Esterbauer et al., 1991; Dianzani et al., 1999).

2.3 Cellular responses and signal transduction

As previously indicated, the adduct formation between HNE and DNA is only one of the several biological effects determined by this aldehyde. Indeed, HNE is considered as a signalling molecule influencing proliferation, differentiation and apoptosis of cancer cells (Dianzani, 2003; Leonarduzzi et al., 2004; Poli et al., 2008; Pizzimenti et al., 2010b). The majority of experimental evidence indicate an antiproliferative role of HNE, when added at low doses (1-10 μM) to cultured cells. The inhibition of proliferation has been observed in leukemic (HL-60, K562, U937, MEL, ML-1) (Barrera et al., 1991; Barrera et al., 1987, Rinaldi et al., 2000; Pizzimenti et al., 2006), neuroblastoma (SK-N-BE) (Laurora et al. 2005), hepatoma (7777, J42) (Muzio et al., 2001; Canuto et al., 1999), osteosarcoma (SaOS2; HOS) (Calonghi et al., 2002; Sunjic et al., 2005), prostate cancer (PC3) (Pettazzoni et al., 2011) cells. This anti-proliferative effect is sustained by the modulation of key genes involved in cell growth control, such as oncogenes (c-myc, c-myb, fos, AP1, cyclins) and anti-oncogenes (pRB, p53, SUFU-1, Mad-1) (Poli et al., 2008; Pizzimenti et al., 2009).

Interestingly, the effect of HNE in normal cell proliferation is more variable if not opposite to that observed in tumor cells. For example, HNE has no effect on normal myeloid stem cells (Hassane et al., 2008) or on human peripheral blood lymphocytes (Semlitsch et al., 2002), while the respective tumour was sensitive to the anti-proliferative effect of aldehyde. On the contrary, in vascular smooth muscle cells 0.1 μM HNE stimulated cell proliferation (Kakishita et al., 2001).

In several cell lines, the inhibition of proliferation was accompanied by apoptosis. The mechanisms of HNE-induced apoptosis through the extrinsic and intrinsic pathways, its self-regulatory role in this process and its interaction with Fas (CD95), p53, and Daxx has been recently reviewed (Awasti et al., 2008).

HNE is also able to induce differentiation, as observed in HL-60, MEL, K562 and SaOS osteosarcoma cells (Barrera et al., 1991; Rinaldi et al., 2000; Calonghi et al., 2002; Cheng et al., 1999; Fazio et al., 1992). Moreover, HNE was shown to induce features of typical differentiated cells, such as chemotaxis (Curzio et al., 1988), phagocytosis and the ability to induce respiratory burst (Barrera et al., 1991) in myeloid cells. HNE also demonstrated the ability to regulate the replicative potential of cells, by inhibiting the telomerase activity. Indeed, in HNE-treated leukemic cells, the expression of the hTERT gene was down-regulated by modulating the expression of transcription factors belonging to the Myc/Mad/Max network (Pizzimenti et al., 2006).

The anti-tumoral properties of HNE are also sustained by the demonstration of anti-angiogenic properties. Stagos and collaborators demonstrated that 5 and 10 μM HNE were able to inhibit the tube formation of human bone marrow endothelial cells (HBMEC) (Stagos et al., 2009). However, conflicting results have been reported, since it has been demonstrated that 1 μM HNE induces an increase of VEGF expression in human retinal pigment epithelial cells (Ayalasomayajula & Kompella, 2002).

The cellular responses to HNE are sustained by affecting cell signalling at multiple levels. Relevant findings in this area have been extensively reviewed (Poli et al., 2008; Leonarduzzi et al., 2004; Dianzani et al., 1999).

In addition to the above cellular responses presented, HNE activates various cytoprotective, stress response pathways, promoting changes in gene expression that facilitate cell survival and recovery from stress (West & Marnett, 2005). For example, HNE activates the transcription factors Nrf2 (Nuclear factor erythroid-derived 2-like 2) and HSF1 (heat shock factor 1), which mediate the antioxidant and heat shock responses, respectively (Jacobs & Marnett, 2007). Nrf2 acts by binding Antioxidant Responsive Elements (ARE) sequences on promoters of certain genes promoting their expression (Thimmulappa et al., 2002). In regard to HNE metabolism, functional ARE sequences have been found on promoter of GST A4 ALDH and ADH (Reddy et al., 2007; Malhotra et al., 2010). Moreover, Nrf2 promotes de novo GSH synthesis by up-regulating expression of the GSH synthesis pathway (Harvey 2009). Nrf2 is controlled by both translational and post-translational mechanisms, in particular the protein Kelch-like ECH-associated protein 1 (KEAP1) mediates Nrf2 ubiquitinaltion followed by proteasomal destruction (Kaspar et al., 2010). In conditions of oxidative stress or in response to many chemicals KEAP1 undergo conformational changes responsible for loss of Nrf2 binding activity. As a consequence Nrf2 can accumulate, translocate in the nucleus and drive expression of the antioxidant program (Reddy et al., 2007).

The heat shock response mediates the induction of a highly conserved set of heat shock proteins (Hsps) (Mosley, 1997). The inducible expression of Hsps is mediated by heat shock transcription factor 1 (HSF1), which translocates to the nucleus upon activation and enhances the expression of genes to form promoters containing heat shoch elements (HSE), such as Hsp70 (Sarge et al., 1993; Baler et al., 1993). A principal function of Hsps is to chaperone other proteins, binding to nascent polypeptide chains as well as to unfolded and damaged proteins. Their function as protein chaperones aids in the recovery of cells from thermal and chemical-induced damage (Hahn & 1982; Howard, 1993). In addition to acting as protein chaperones, Hsps inhibit cell death by directly inhibiting a variety of pro-apoptotic mediators, such as HNE (Jacobs et al., 2007).

It is very likely that the majority of effects observed on cell signalling and cellular responses can be mediated by the reaction of HNE to proteins and peptides. Quantitatively, proteins and, among peptides, the GSH, represent the most important group of HNE-targeted biomolecules. It was estimated that 1–8% of the HNE formed in cells will modify proteins (Siems & Grune, 2003). Most of the identified targets are enzymes, carriers, receptors, ion channels, transport proteins, cytoskeletal, heat shock proteins and others. The biological significance of the HNE-protein adducts identified have been reviewed by several authors (Uchida, 2003; Poli et al., 2008). Some of the protein-adducts identified can explain the anti-tumoral effect exerced by this aldehyde. For example, it was demonstrated that the inhibition of cell proliferation in the human colorectal carcinoma cell line (RKO) and human lung carcinoma cell line (H1299) by HNE was mediated by the direct reaction of HNE with

IκB kinase (IKK), the key enzyme regulating the NF-κB activation (Ji et al., 2001b). Moreover the HNE adducts with alpha-enolase, at the cellular surface of leukemic cells, suggest a new role for HNE in the control of tumour growth and invasion, since HNE causes a dose- and time-dependent reduction of the plasminogen binding to alpha-enolase. As a consequence, HNE reduces adhesion of HL-60 cells to HUVECs (human umbilical vein endothelial cells) (Gentile et al., 2009).
New perspectives of HNE role in cancer-inducing signaling pathways have recently emerged, by recent findings on microRNA (miRNA) (Pizzimenti et al., 2009), a class of conserved non-coding small RNAs, which regulate gene expression by translation repression of coding mRNAs (Bartel, 2004).

2.4 HNE content in human cancers

Several studies on human cancer tissues have analysed the HNE or HNE-protein adduct content, in order to find a possible correlation between this marker of lipid peroxidation and the progression of cancer.
HNE content has been reported to increase along with the progression of breast cancer (Karihtala et al., 2011) and astrocytoma (Zarkovic et al., 2005). In human renal cell carcinoma, immunohistochemistry for HNE-modified proteins showed positive staining in the cytoplasm of tumor cells, with respect to controls, without correlation to the clinical stage (Okamoto et al., 1994).
However other reports have demonstrated the opposite: a low or undetectable lipid peroxidation, as well as HNE content, such as in hepatomas (Dianzani, 1993).
Several studies have shown elevated lipid peroxidation markers in the sera, plasma or urine of breast carcinoma (Hung et al., 1999; Chandramathi et al., 2009), cervical intraepithelial neoplasia and carcinoma of the cervix (Looi et al., 2008), head and neck squamous cell carcinoma (Gupta et al., 2009) and prostate tumor (Kotrikadze et al., 2008), compared to healthy controls. However, these extratumoral measurement are likely, at least partly, to reflect generalized oxidative stress and /or inflammation in the whole body.

3. HNE and colorectal carcinogenesis

3.1 Sources and fate of HNE in colon

Colon cells can be exposed to HNE derived form different sources (Figure 1). It is possible to find HNE directly in the food (Gasc et al., 2007), since it can derived from lipid peroxidation of PUFAs introduced with diet or from endogenous PUFAs presents in cellular membranes.
A small amount of HNE can reach the colon also via bile. Following a single intravenous administration of [3H]-HNE, five metabolites were present in the bile, namely GSH–HNE, GSH–DHN, DHN, and HNA-lactone mercapturic acid conjugates (Laurent et al., 1999). Within 4 hr from injection of the radiolabel 3[H]-HNE, 19.5% of the injected radioactivity was found in the bile, whereas only 3% was found in the feces within 48 hr (Laurent et al., 1999). The existence of an enterohepatic circulation for HNE metabolites has been unequivocally demonstrated (Laurent et al., 1999) using a model linking donor rats (injected intravenously with [4-3H]HNE) and recipient rats (to which the bile from donor rats was delivered intraduodenaly). This enterohepatic circulation, approximately 8% of the total dose, may explain the low amount of radioactivity recovered from faeces when rat were dosed intravenously with [4-3H]HNE.

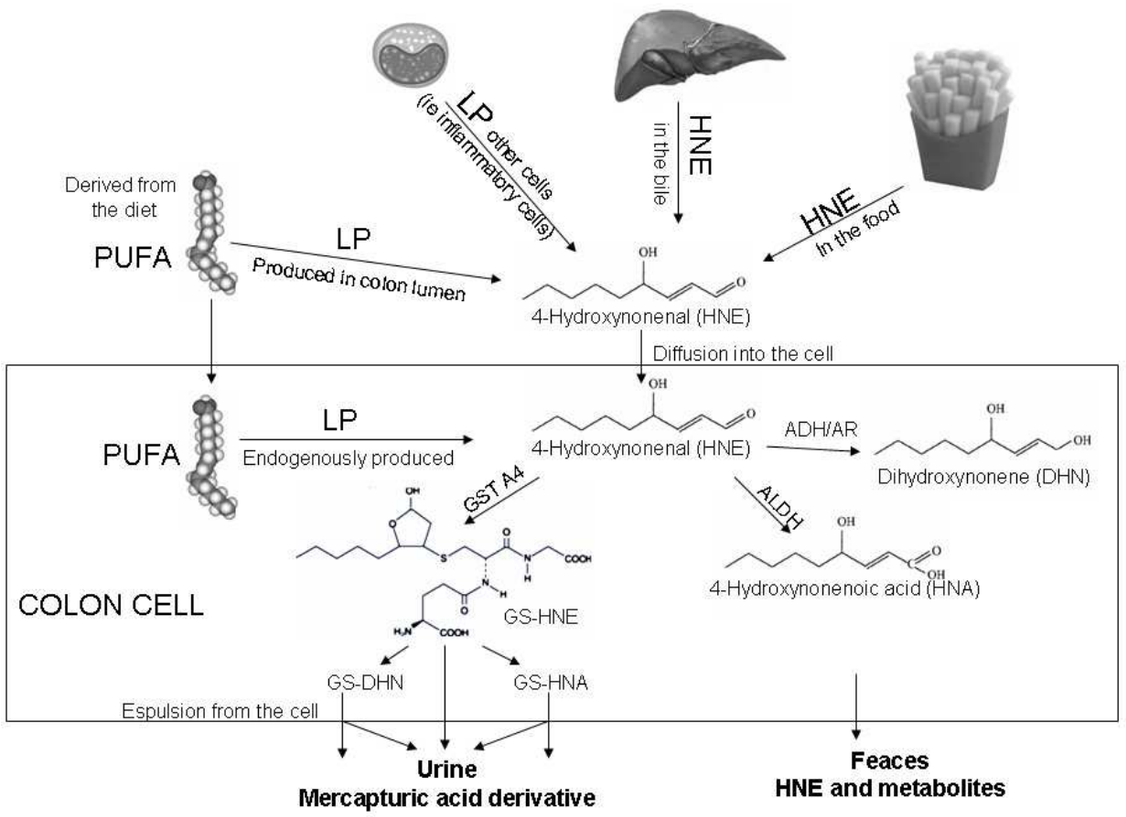


Fig. 1. Sources and fate of HNE in colon

Metabolic transformation of HNE starts in enterocites, where GSH-HNE is the main metabolite produced (Grune et al., 1991). The majority of HNE metabolites are found in the urine. Indeed, following the intravenous administration of [3H]-HNE in rats, 67%, 3%, 0.16%, and 6.5% of the injected radioactive dose was recovered from urine, faeces, liver and remaining tissues, respectively (Alary et al., 2003). The urinary HNE metabolites were separated by HPLC and the resolved peaks were identified as mercapturic acid conjugates of HNA, DHN, HNE and HNA-lactone, where DHN-MA, and to a lesser extent HNA lactone-MA, have been found to be the major urinary metabolites of HNE in rats (Boon et al., 1999). DHN-MA has been confirmed to be the major urinary HNE metabolite also in human urine (Alary et al., 1998).

The microflora of the human intestine can also affect levels of lipid peroxidation, since the antioxidative effect of lactic acid bacteria has been demonstrated (Lin et al., 1999). In particular, the antioxidative activity of Bifidobacterium longum ATCC 15708 and Lactobacillus acidophilus ATCC 4356 was measured based on the inhibition of linoleic acid peroxidation. Both intact cells and intracellular cell-free extracts of B. longum and L. acidophilus demonstrated an antioxidative effect on inhibiting lipid peroxidation. The antioxidative activity ranged from 38 to 48% inhibition of linoleic acid peroxidation. This indicates that B. longum and L. acidophilus have a very strong antioxidative effect on inhibiting lipid peroxidation (Lin et al., 1999).

Low level of HNE and its metabolites can be found also in faecal water (Alary et al., 2003) and numerous studies have emphasized the lipid peroxidation products of faecal water in colon cancer as diet-related factors (Lapre et al., 1992; Glinghammar et al., 1997).

3.2 Pro-tumoral and anti-tumoral role of HNE in colon carcinogenesis

Several authors have reported evidence that sustains the pro-tumoral activity of HNE and other products of lipid peroxidation in colon carcinogenesis. These findings include in vitro (see table 1) and in vivo studies, which demonstrate the genotoxic properties of HNE on coloncarcinoma cell lines, the increase of HNE content along with the progression of colorectal cancer and the increase of HNE-DNA adducts in vivo. However other studies, seem to demonstrate the opposite (see table 1). Consistent with the hypothesis of an anti-tumoral role of HNE are the results showing the inhibition of cell growth, the induction of apoptosis in several colon cancer cell lines, as well as the demonstration that HNE content decreases in biopsies of colon-cancer tissues with respect to normal mucosa. A deeper discussion of these opposite results is here reported.

COLON CANCER CELL LINE	HNE DOSE	OBSERVED EFFECTS	PROTEIN / PATWAY INVOLVED / MAIN RESULTS	REFERENCE
CaCo-2	1 μM	apoptosis, ROS production, enhanced by co-treatement with TGF-β1	HNE activates c-Jun N-terminal kinase (JNK) and Smad4, effects enhanced by co-treatment with TGF-β1	Zanettiet al., 2003; Vizio et al., 2005; Biasi et al., 2006
CaCo-2, HT-29	1 μM	cell growth inhibition, apoptosis	HNE down-regulates telomerase activity and hTERT expression, through modulation of Myc/Mad/Max network	Pizzimenti et al., 2010
CaCo-2	1 μM	cell growth inhibition, apoptosis	HNE induces an increase of c-myc expression and a subsequent down-regulation; HNE increases bax and p-21 expression	Cerbone et al., 2007
RKO	30-75 μM	apoptosis	HNE activates a mitochondrion-dependent pathway, involving cytochrome c release and caspase activation	Ji et al., 2001a
RKO	40 μM	apoptosis	HNE inhibits NF-kB activation by direct interaction with IkB kinase (IKK)	Ji et al., 2001b
RKO	30-60 μM	apoptosis	comparison with other aldehydes producted during lipid peroxidation (HPNE,ONE) and stereoisomers of HNE	West et al., 2004
RKO	5, 20, or 60 μM	5 and 20 μM subcytotoxic, 60 μM apoptosis	by using microarray technology, HNE simultaneously affects multiple stress signaling pathways	West et al., 2005

COLON CANCER CELL LINE	HNE DOSE	OBSERVED EFFECTS	PROTEIN / PATWAY INVOLVED / MAIN RESULTS	REFERENCE
RKO	30-60 µM	apoptosis	beside the activation of pro-apoptotic pathway, HNE activates a protective signal activation through activation of HSF1, Hsp70-1 and Hsp40 and stabilization of Bcl-XL	Jacobs & Marnett, 2007
RKO	45 µM	apoptosis	BAG3, induced by HSF-1, increases cell survival, by stabilizing the level of Bcl-2 family proteins	Jacobs & Marnett, 2009
HCT15	20-80 µM	cell death	AKR1B10-overexpressing cells are resistant to cytotoxicity of HNE	Matsunaga et al., 2011
Apc+/+, Apc+/- colon epithelial cells	10-250 µM	cell death	HNE reduces cellular viability of either Apc+/+ and Apc-/+ cells, with lesser extent in Apc-/+ cells	Pierre et al., 2007
CaCo-2		cell death	HNE increases prostaglandin E2 (PGE2) production and cyclooxygenase (COX)-2 expression; inhibition of AR prevented HNE-induced effects	Tammali et al., 2006
HT-29, HT-29clone19A	100-200 µM	genotoxicity	butyrate reduces DNA damage caused by HNE, through induction of Glutathione S-Transferase	Ebert et al., 2001
primary human colon cells, LT97, HT-29clone19A	100-250 µM	genotoxicity	HNE induces TP53 specific DNA damage	Schaeferhenrich et al., 2003
HT-29	150 µM	genotoxicity	Two fermentation products of wheat bran reduce the genotoxicity of HNE, via up-regulation of the activity of GSTs	Glei et al., 2006
primary human colon cells, LT97	0-250 µM	genotoxicity	HNE induces DNA damage on specific genes (APC,TP53, KRAS)	Glei et al., 2007
HT-29	100-250 µM	genotoxicity	butyrate induces resistance to HNE damage, by inducing GSH syntesis and increasing GSTA4-4 level	Knoll et al., 2005, Scharlau et al., 2009

Table 1. HNE in vitro effects on colon cancer cell lines

3.2.1 HNE-DNA adducts in colon and colon cancer

HNE-dG adducts, were found in normal human colon tissue, as well as DNA adducts with other lipid peroxidation products, such as acrolein and MDA (Chung et al., 2000). The levels of HNE-dG in tissue DNA examined so far are estimated to be in the range of 3-9 adducts per billion bases (3-9 nmol/mol guanine) (Chung et al,. 2000).

The etheno-DNA adducts, inter alia formed from epoxidized HNE, were found at increased level in colonic polyps of familial adenomatous polyposis (FAP) patients. Mean adduct levels in FAP polyps were 65 εdA/109 and 59 εdC/109 parent nucleotides, being 2 to 3 times higher than in unaffected colon tissue (Schmid et al., 2000). Interestingly, the level of etheno-DNA adducts in colon carcinoma tissues were found to be similar to unaffected colon (Schmid et al., 2000), suggesting a possible HNE role in the early events of colon carcinogenesis.

On the contrary, Obtułowicz and collaborators (2010) have found that, in colon cancer patients, the DNA-HNE adducts εdA and εdG, measured both in colon tissues and blood leukocytes, were lower in patients than in controls (Obtułowicz et al., 2010). These authors have measured the two corresponding metabolites also, 1,N6- Ethenoadenine (εAde) and 3,N4-ethenocytosine (εCyt), catalized by BER, the major pathway of etheno adduct elimination from DNA (Obtułowicz et al., 2010). Both excision activities were significantly higher in tumor than in normal colon tissues and this feature could be explained by the increased level of abasic site endonuclease (APE1), belonging to BER system, in coloncancer patients with respect to controls (Obtułowicz et al., 2010).

A possible pro-cancinogenic role of etheno-DNA adducts is also sustained by the finding that in the colon of patients with inflammatory bowel disease εdC, but not εdA, are increased. In particular it has been demonstrated that εdC was 19-fold higher in colonic mucosa of Crohn's disease and 4-fold higher in the colonic mucosa of ulcerative colitis patients, when compared to normal tissues (Nair et al., 2006). Since patients with ulcerative colitis (UC), and Crohn's (CD) have an elevated risk for developing colon cancer (Konner et al., 2003), the authors suggest that the promutagenic etheno-DNA adducts, generated as a consequence of chronic inflammation, can act as a driving force to malignancy in cancer-prone inflammatory diseases (Nair et al., 2006).

HNE can also contribute to induce colon carcinogenesis, by inhibiting the DNA repair mechanism of such adducts. Indeed, Feng and collaborators (2004) demonstrated that 50 μM HNE inhibits NER in the human colon epithelial cell line HCT116. The repair capacity for benzo[a]pyrene diol-epoxide and UV light-induced DNA damage was greatly compromised in cells treated with HNE.

3.2.2 Genotoxicity and mutagenicity of HNE in colon cancer

Comet assay demonstrated that HNE, at concentration higher than 150 μM, displays a genotoxic effect in the colon carcinoma cell line HT-29 (Glei et al., 2006; Ebert et al., 2001; Knoll et al., 2005) and in HT29clone19A, a permanently differentiated sub-clone treated with sodium butyrate (Augeron & Laboisse, 1984). Moreover, such high doses of HNE were able to affect DNA integrity in primary human colon cells (Schaferhenrich et al., 2003; Glei et al., 2007) and in LT97, an established cell line derived from a differentiated microadenoma, representing a model of an early premalignant genotype, carrying adenomatous polyposis coli (APC) and Ki-ras mutated, but normal p53 (Richter et al., 2002), three well-characterized genes involved in coloncancer progression (Fearon et al., 1990).

Genotoxicity of HNE is higly dependent on cellular GSH level. Indeed, GSH depletion leads to and increase of HNE genotoxicity in the HT-29 colon carcinoma cell line (Knoll et al., 2005). Moreover, HNE displayed a higher genotoxicity in LT97 than in HT29clone19A and primary human colon cells. This result can be explained by the lower GST expression found in LT97 compared to HT29clone19A and primary human colon cells (Schaferhenrich et al., 2003).

Recently, by using a refined comet assay (Comet-FISH) (Glei et al., 2009), which combined the classical comet assay with the fluorescence in situ hybridisation, it has been demonstrated that HNE concentrations higher than 150 µM were able to affect DNA integrity on the p53 (Schaferhenrich et al., 2003; Glei et al., 2007), Ki-Ras and APC genes (Glei et al., 2007), in primary human colon cells and the colon adenoma cell LT97. After cell incubation with HNE, the p53 gene, the crucial target gene for the progression of adenoma to carcinoma, migrated more efficiently into the comet tail than the global DNA, indicating a high susceptibility of the p53 gene to HNE (Glei et al., 2007). Moreover, the TP53 gene sensitivity to the DNA damage induced by HNE was significantly higher with respect to APC and KRAS genes. This particular sensitivity is especially apparent in LT97 cells (Glei et al., 2007). This may be due to the fact that LT97 cells normally carry damaged APC and KRAS, but undamaged TP53 (Richter et al., 2002). In normal colonocytes, APC and KRAS were also sensitive to damage (Glei et al., 2007). These findings are highly interesting when considering the sequence of mutational events that occur during human colon carcinogenesis (Vogelstein et al., 1988). APC and KRAS mutations transform normal epithelial (stem) cells into initiated, more rapidly proliferating cells to yield dysplasia and small adenoma. TP53 mutations in adenoma are then crucial alterations leading to further progression and to carcinoma. Based on studies of Glei and collaborators (2007), it is possible to conclude that HNE could potentially contribute to both cancer initiation and progression in the colon, if produced in sufficient amounts. However, as mentioned in the previously chapter, it is unlikely that HNE is reaching such high concentrations (150 µM) in colon in vivo. Moreover it still remains to be studied to what extent the observed genotoxicity of HNE is related to mutagenicity. Consistent with this hypothesis, as previously reported, it has been demonstrated that 50 µM HNE treatment in human TK-6 lymphoblastoid cell line induces a high frequency of G.C to T.A mutations at the third base of codon 249 (AGG*) of the p53 gene (Hussain et al., 2000), a mutational hot spot in human cancers, particularly in hepatocellular carcinoma (Hsu et al., 1991). The adduct of HNE to codon 249 of the p53 gene has been also found by Hu and collaborators (2002). These authors exposed DNA of exons 5, 7 and 8 of human p53 gene, where the large majority of p53 mutations occur, to a very high concentration of HNE (192 mM or more). They identified two main HNE adducts, the first already mentioned at codon 249 (exon 7) and the second at codon 174 (exon 5) (Hu et al., 2002). However, the possible contribution of HNE to p53 mutations, through the formation of DNA adducts remains to demonstrated, since codon 249 and codon 174 of p53 usually are not mutated in colorectal rectal. Indeed, mutations at codon 175, 245, 248, 273, and 282 account for approximately 43% of all p53 mutations in CRC (Soong et al., 2000; Soussi et al., 2000; Soussi & Beroud, 2003).

3.2.3 HNE role in controlling cell proliferation, apoptosis of colon cancer cell

Several findings have been collected through the years related to the anti-proliferative and pro-apoptotic in colon cancer cells. These results, even obtained with very low doses of

HNE, easily reacheable in vivo, cast doubt on the pro-tumoral HNE role. 1 μM HNE is able to inhibit cell proliferation of Caco-2 and HT-29 colon cancer cells (Cerbone et al., 2007; Pizzimenti et al., 2010b; Vizio et al., 2005) and concentrations ranging form 1 to 100 μM are able to induce apoptosis in Caco-2, HT-29, RKO, HCT15 colon cancer cells. (see table I for references). A number of genes or cell signalling pathways have been found to be affected by HNE, and their modulation can explain the biological effects observed.

Results obtained in our laboratories demonstrated that the inhibition of proliferation in Caco-2 and HT-29 colon carcinoma cells by 1 μM HNE is sustained by the down-regulation of telomerase activity and hTERT expression, the catalytic subunit of telomerase (Pizzimenti et al., 2010b). The major mechanism of HNE action seems to be the modulation of expression and activity of transcription factors belonging to the Myc/Mad/Max network (Pizzimenti et al., 2010b).

After HNE treatment, apoptosis of several colon cancer cell lines was investigated by different authors and different pathways were considered to be involved. In Caco-2 human colon adenocarcinoma cell line, 1 μM HNE caused an increase of bax expression (Cerbone et al., 2007) and the apoptosis induction is mediated by JNK activation. Indeed, the HNE-mediated apoptotic cell death was significantly prevented by preincubating the cells with the selective JNK inhibitor SP600125 (Biasi et al., 2006).

Ji and collaborators investigated the mechanism of HNE-induced cell death in human colorectal carcinoma cells and found that HNE-induced apoptosis depends on alteration of mitochondrial function, leading to the release of cytochrome c and subsequent activation of caspase cascade (Ji et al., 2001a). The authors have further demonstrated that HNE inhibited IκB kinase activity by direct interaction with IκB kinase and suggested that HNE is an endogenous inhibitor of NF-κB activation that acts by preventing IκB kinase activation and subsequent IκB degradation (Ji et al., 2001b).

The molecular mechanism of HNE induced apoptosis was investigated in RKO colon cancer cells also. In this cell line, beside the pro-apoptotic stimuli, HNE activates the stress response pathways, that abrogate programmed cell death. Moreover, HNE elicits the nuclear translocation of HSF1 and promotes Hsp40 and Hsp72 expression (Jacobs & Marnett, 2007). The silencing of HSF1 sensitizes the colon cancer cells to HNE-induced apoptosis, through a mechanism involving the control of BCL-XL, BAG3 protein turnover (Jacobs & Marnett, 2007; Jacobs & Marnett, 2009)

3.2.4 HNE content in human colon cancers

Only a few studies have investigated the level of the lipid peroxidation products, in particular HNE, in human colon cancers and results are contradictory. It has been demonstrated that the levels of proteins modified by HNE and MDA in colorectal cancer tissues were significantly increased (Murawaki et al., 2008). By immunohystochemical analysis, Murawaki and collaborators (2008) have demonstrated that the proteins modified by HNE were stained diffusely in the cytoplasm of cancer cells, while they were weakly stained in normal tissues. Similar results have been obtained by Kondo and collaborators (1999). Immunostaining of HNE-histidine adducts was observed in the cytoplasm of colon cancer tissues. Immunoreactivity was also found in the cytosol of infiltrating inflammatory cells. Western blot analysis of HNE-histidine adducts confirmed the results, since larger amounts of modified proteins were detected in carcinomas than in nontumorous epithelial counterparts (Kondo et al., 1999). The authors also demonstrated that HNE content

increased along with the progression of colorectal cancer, since tubular adenoma cells revealed a weaker staining, similar to the staining of non-tumorous epithelial cells (Kondo et al., 1999). An increase of HNE content in colon cancer tissues have been found also by Skrzydlewska and collaborators (2005). These authors analyzed the HNE content in homogenates of human colon cancer tissues, by measuring HNE as a fluorimetric derivative. These authors have demonstrated that the level of HNE was significantly increased ($P<0.001$) in cancer tissue compared to control group, with highest in G3-grade adenocarcinoma and mucinous adenocarcinoma and clinical IV stage of colorectal cancer.
In contrast with these results, other scientists demonstrated a decrease of HNE in colon cancer tissues. Indeed, it was demonstrated that HNE was significantly decreased in cancer specimens, with respect to normal tissues, by measuring the HNE content in tissue biopsies from patients with colon adenocarcinoma of different TNM and G stage (Biasi et al., 2002; Zanetti et al., 2003). This result was confirmed later by the same group (Biasi et al., 2006). Moreover, Chiarpotto and collaborators (1997) have demonstrated that the fluorescent adducts with plasma proteins and HNE were significantly lower in the plasma from cancer patients (all stage G3, pT3pN0) than in controls.

3.2.5 HNE metabolism in colon cancer

In colon cells, the enzymes of HNE metabolism are present. Staining with anti GST A4 specific antibodies revealed a significant expression of GST A4 in columnar and crypt epithelial cells of normal colon mucosae (Desmots et al., 2001), as well as in colon cancer cell lines (Scharmach et al., 2009; Knoll et al., 2005). Moreover, both the oxidative and reductive metabolisms of HNE are well represented in colon cells, since both ALDH or ADH have been found to be significantly expressed in colon mucosae (Seitz et al., 1996; Yin et al., 1994). The expression of AR is also enhanced in various forms of cancer, such as hepatoma (Zeindl-Eberhart et al., 1997) and melanoma cancer (Kawamura et al., 1999).
By affecting HNE metabolism enzymes, it is possibly to modulate the HNE concentration inside cells. This could be critical for cancer growth regulation or DNA genotoxicity. Indeed, butyrate, produced during gut fermentation, has a chemoprotective role toward HNE injury, when added at high concentration, such as 100-200 µM in HT-29 colon cancer cells (Knoll et al., 2005). The chemoprotective effect of butyrate seems to be related to the increasing the expression of glutathione S-transferases GSTP1 (Ebert et al., 2001) and hGSTA4-4 (Knoll et al., 2005) able to catalyze the conjugation of HNE with glutathione. Similar results were obtained in HT-29 cells by using two wheat bran-derived arabinoxylans, fermented under anaerobic conditions in human feces. These two fermentation products inhibited growth and reduced the genotoxicity of HNE (100-200 µM) via up-regulation of the activity of GSTs, in absence of a GSTP1 or hGSTA4-4 increase (Glei et al., 2006).
There is a growing interest in targeting aldose reductase (AR), as a novel therapeutic approach in preventing progression of colon cancer (Tammali et al., 2011). AR besides reducing aldo-sugars efficiently reduces toxic lipid aldehydes and their conjugates with glutathione (Tammali et al., 2006). Indeed, inhibition of AR by sorbinil or by antisense ablation, prevented FGF–induced and PDGF–induced proliferation of Caco-2 cells at S-phase (Tammali et al., 2006). Similar results were also obtained in other colon cancer cell lines, by Ramana and collaborators which show that the inhibition of AR prevents epidermal growth factor (EGF)– and basic fibroblast growth factor (bFGF)–induced HT29,

and cell proliferation, by accumulating cells at the G1 phase of the cell cycle, through the AKT/Phosphoinositide 3-Kinase/E2F-1 pathway. Analogous results were obtained in SW480 and HCT-116 colon cancer cells (Ramana et al., 2010).

More interestingly, in vivo studies showed that administration of aldose reductase-small interfering RNA (siRNA), or the AR inhibitor fidarest, to nude mice bearing SW480 human colon adenocarcinoma cells, led to a complete arrest of tumor progression. Such evidence suggests a key role for aldose reductase in growth factor-induced proliferation in colon cancer cells and it points to inhibition of aldose reductase as a novel therapeutic approach in preventing progression of colon cancer (Tammali et al., 2006; Ramana et al., 2010).

Recently, the ATP-depent transporter RLIP76 (Ral binding protein1) has been considered for its role in controlling HNE content inside the cells. Indeed, it has been demonstrated that this transporter with multi-specific transport activity towards glutathione-conjugates and chemotherapeutic agents, is also specific for GSH-HNE (Sharma et al., 2002). The expulsion of GS-HNE from cells represents another critical step in HNE detoxification since it avoids the accumulation of adducted GSH and permits the restoration of GSH/GSSG equilibrium. RLIP76 protein is frequently overexpressed in cancer lesions (Vatsyayan et al., 2010), included colon cancers (Singhal et al., 2007), thus there is a growing interest in considering this protein as target in cancer therapy (Vatsyayan et al., 2010). When RLIP76 is inhibited, a rapid increase in HNE-GSH is observed, both in vitro (Awasthi et al., 2003; Cheng et al., 2001; Yang et al., 2003) and in vivo (Vatsyayan et al., 2010). Recent studies show that the inhibition and/or depletion of RLIP76 by antibodies, siRNA, or antisense can lead to a drastic and sustained regression of lung, kidney, melanoma, prostate, and colon cancer xenografts with no observed recurrence of tumors (Vatsyayan et al., 2010). In particular, it has been shown that xenografts of SW480 human colon cancer cells in nude mice can be completely regressed by anti-RalBP1 immunoglubulin G or by suppression of RalBP1 expression using phosphorothioate antisense against it (Singhal et al., 2007).

The super family of aldo–keto reductase (AKR) enzymes seems to be involved in tumor development, and growing evidence is accumulating, suggesting them as a new class of tumor marker. These enzymes are hydroxysteroid dehydrogenases with a broad substrate specificity for other carbonyl compounds including HNE. The isoform AKR1B10 seems to be particulary involved in the transformation of HNE to the oxidized counterpart 4-oxonon-2-enal (4-ONE) (Martin et al., 2009). AKR1B10 is also up-regulated in many types of solid tumors (Fukumoto et al., 2005; Yoshitake et al., 2007; Breton et al., 2008; Satow et al., 2010), and its gene silencing results in growth inhibition of colorectal cancer cells (Yan et al., 2007), as well as in increasing HNE-elicited cell death (Matsunaga et al., 2011).

Recently, some family members of AKR enzymes have been shown to be overexpressed and linked to resistance against anticancer drugs such as anthracyclines, cisplatin, and methotrexate (Veitch et al., 2009; Cheng al., 2008; Selga et al., 2008). As regarding colon cancer, experimental data suggest that the up-regulation of AKR1B10 was related with acquisition of resistance to the anticancer drug mitomycin-c (MMC) in HT-29 colon cancer cells (Matsunaga et al., 2011). The cytotoxic effects of MMC seems to be mediated by the formation of HNE. Thus, the biological significance of the increasing of AKR1B10 in MCC resistant cancer cells would be an ability to better detoxify cytotoxic aldehydes including HNE. (Matsunaga et al., 2011). In the resistant cells, treatment with an AKR1B10 inhibitor decreased their MMC tolerance (Matsunaga et al., 2011), suggesting its use as adjuvant therapy in drug resistant cells, in which AKR1B10 is over-expressed.

Many dietary cancer chemopreventive compounds, such as cruciferous vegetables, could activate the antioxidant responsive element (ARE), a critical regulatory element in the promoter sequence of genes encoding cellular Phase II detoxifying and antioxidant enzymes. Transcriptional activation of ARE is typically mediated by the transcription Nuclear factor-erythroid 2-related factor 2 (Nrf2). Thus, this transcription factor has emerged as a novel target for the prevention of colon cancer (Saw & Kong, 2011). However, stable RNAi-mediated knockdown of Nrf2 in human colon cancer cells suppressed tumor growth in mouse xenograft settings and colon tumor angiogenesis by inhibiting Hypoxia-Induced Activation of HIF-1a (Kim et al., 2011). Thus, the role of Nfr2 in colon carcinogenesis still has to be explored.

3.2.6 HNE and nutrition

It is well accepted that development and progression of colon cancer is generally associated with lifestyle-dependent risk factors, such as dietary choices (Pearson et al., 2009). HNE can be directly found in food (Gasc et al., 2007) or its production can be enhanced by the presence of some nutrients, i.e. ω-6 PUFAs, or some fermentation products of diet, i.e. butyrate, can modulate the metabolism of this aldehyde, thus modifying its concentration. In this context, it is very interesting to explore the connection between HNE, nutrition and colon carcinogenesis.

HNE has been founded in different foods, correlating with the amount of ω-6 (Surh et al., 2010). UsingGC–MS technology, scientists measured 4-hydroxy-alkenaks content in vegetable oils, fish and shellfish, calculating the HNE dietary intake of the Korean population (Surh et al., 2005). Korean daily exposure to 4-hydroxy- 2-alkenals was found to be of 4.3 mg/day and HNE was found to be more represented (2.7 mg). There was an additional exposure to more than 11.8 mg/day 4-hydroxy-2-alkenal from fried foods. The combined exposure would be, therefore, 16.1 mg/day corresponding to 0.3 mg/kg body weight/day for a 60 kg Korean adult. Additionally, the screening of PUFA-fortified foods including infant formulas and baby foods commercially available on the Korean markets were screened, and it was estimated that 3- month to 1-year-old babies sticking exclusively to these products could be exposed to a maximum 20.2 μg/kg BW/ day of 4-hydroxy-2-alkenals (Surh et al., 2007). However, in spite of the biological toxicity of 4-hydroxy-2-alkenals, the risk for humans cannot be quantified due to the lack of a virtually safe dose of the compound (Surh et al., 2005).

A diet high in red and processed meats can increase colon cancer risk by 12–20%. The mechanism of promotion by haem iron is not known, but may be linked to oxidative stress and subsequent events such as lipid pro-oxidation and HNE production (Sesink et al., 1999; Sawa et al., 1998). Indeed, the dietary haem, in the form of either haemoglobin or meat, promotes precancerous lesions, aberrant crypt foci (ACF) and mucin-depleted foci in the colon of rats (Pierre et al., 2003; Pierre et al., 2004). This haem-induced promotion was associated with increased lipid peroxidation in faecal water and strong cytotoxycity activity of faecal water on the cancerous colonic epithelial cell line (Pierre et al., 2003). Further, Pierre and collaborators (2007), have explored the effect of faecal water components of haem-fed rats, on normal APC +/+ or premalignant APC -/+ cells, demonstrating that the toxic effects observed correlated with the presence of HNE in the faeces. Moreover, the premalignat APC -/+ cells were more resistant to apoptosis with respect to normal APC +/+. The authors suggested, thus, that the premalignant mutation confer to cells the resistance to the

inhibitory signal, allowing them to undergo further mutations and follow a tumoural pathway (Pierre et al., 2007).

In a randomized human study, the urinary excretion of DHN-MA, the major metabolite of HNE detectable in urine was compared in volunteers consuming different levels of heme iron. The volunteers fed with a low red meat diet (60 g/day) showed a twofold increase of DHN-MA when supplemented with heme iron as blood sausage (70 g/day). Since colon preneoplastic lesions and DHN-MA excretion in the experimental animal were clearly associated with dietary heme iron, urinary DHN-MA was suggested as a promising biomarker of colon carcinogenesis (Pierre et al., 2006).

The role of fat present in the diet in coloncarcinogenesis has been explored by several authors and comprehensive reviews have been published. In particular, diets rich in ω-6 PUFAs, contained in vegetable oils, seem to enhance the development of colon tumors, whereas ω-3 PUFA-containing diets, such as fish oil, reduce colon cancer incidence (Reddy, 2002; Kim & Milner, 2007). Thus, it is possible to suggest a putative HNE role in colon carcinogenesis, since HNE is derived from peroxidation of ω-6. However, the complexity of the issue forces us to be more cautious. Indeed, Eder and collaborators investigated the impact of different fatty-acids composition in the diet on cancer development, measuring the formation of the promutagenic HNE-dG in the mucosa of several organs, such as colon. The correlation between adduct levels and the different fatty acids assumption was not uniform for all organs and they didn't find a clear relationship between fatty acids and adduct levels in the colon (Eder et al., 2008). Moreover, beside lipid peroxidation products it is necessary to consider the eicosanoids, also derived from PUFAs. Indeed, eicosanoids have different properties in cancer cell growth, invasion and angiogenesis when derived from ω-6 or ω-3 fatty acids (Berquim et al., 2008), thus suggesting a role in carcinogenesis.

Epidemiological studies show a reduction in risk for individuals and populations consuming high amounts of vegetables. The protective effect of vegetables may be due to their content of complex carbohydrates such as dietary fiber and starch (Scheppach et al., 1999). A substantial amount of starch escapes digestion in the small intestine (Englyst et al., 1992) and this fraction is called enzyme-resistant starch (RS). Starch and dietary fiber together are the principal substrates controlling the pattern of fermentation in the colon and, thus, the metabolism of compounds, like bile acids, nitrate and enzyme activityes (bacterial and antioxidant enzymes), which have been implicated in carcinogenesis. The effect of enzyme-resistant starch (RS) on the development of colon cancer was reported to include both chemopreventive and tumorigenic activity in humans. Indeed, an inverse association between starch consumption and large bowel cancer incidence has been found in an international comparison in 12 populations worldwide (Cassidy et al., 1994). However, an increased cancer risk with high-starch intake has been also reported (Franceschi et al., 1998; Favero et al., 1999). Wacker and collaborators (2002) have studied the number of 1,N2-propanodeoxyguanosine-30- monophosphate (HNE-dGp) adducts in the colonic mucosa of volunteers fed with starchy foods enriched with a highly resistant amylomaize starch (Hylon VII) and they found an increase of the HNE-dGp adduct, whereas there was no evidence for an increased cell proliferation in the upper crypt.

Finally, as already mentioned, nutrients can modulate the HNE level in the colon, by affecting its metabolism. This is the case of fermented products of diet, such as butyrate (Knoll et al., 2005; Glei et al., 2006) and wheat bran-derived arabinoxylans, that can affect the HNE levels, by upregulating GSTs activities.

4. Conclusion

Lipid peroxidation is a physiological and pathological process that elicits a number of electrophilic compounds able to modulate several cellular processes. Among these, HNE is the most studied aldehyde, due to its high biological activity. Since HNE is a normal constituent of the diet or can be produced in the gut, colon cells can be exposed to this aldehyde.

Low doses of HNE are able to inhibit cell proliferation and induce differentiation of colon cancer cells. Conversely, a high concentration of HNE exhibits genotoxic and mutagenic activity. We believe that the concentration of HNE and other lipid peroxidation products in the colon, represent a steady state level between production and catabolism. The alteration of this equilibrium elicits a stress condition for colon cells and, possibly, could be involved in colon carcinogenesis, although there is no scientific consensus in supporting its pro-tumoral action.

Results on HNE content in human biopsies of coloncancer tissues are contradictory, and the positive correlation between HNE content and cancer progression doesn't allow an assumption whether the HNE increase during the progression of colon cancer may represent a cause or a consequence of this process. However, in colon cancer cells, HNE induces apoptosis and telomerase inhibition. Thus, we can hypothesize that HNE, produced during radiotherapy or chemotherapy, can participate to the control of tumor growth and tumor cell death.

5. References

Acharya, A., Das, I., Chandhok, D. & Saha, T. (2010). Redox regulation in cancer: a double-edged sword with therapeutic potential. Oxidative Medicine and Cellular Longevity, Vol.3, No.1, (January 2010), pp. 23-34, ISSN 1942-0900

Alary, J., Debrauwer, L., Fernandez, Y., Cravedi, J.P., Rao D. & Bories, G. (1998). 1,4-Dihydroxynonene mercapturic acid, the major end metabolite of exogenous 4-hydroxy-2-nonenal, is a physiological component of rat and human urine. Chemical Research in Toxicology, Vol. 11, No.2, (February 1998), pp. 130–135, ISSN 0893-228X

Alary, J., Gueraud, F. & Cravedi, J.P. (2003). Fate of 4-hydroxynonenal in vivo: Disposition and metabolic pathways. Molecular Aspects in Medicine, Vol.24, No.4-5, (August-October 2003), pp. 177–187, ISSN 0098-2997

Augeron, C. & Laboisse C.L. (1984). Emergence of permanently differentiated cell clones in a human colonic cancer cell line in culture after treatment with sodium butyrate. Cancer Research, Vol.44, No.9, (September 1984), pp. 3961–3969, ISSN 0008-5472

Awasthi, S., Singhal, S.S., Singhal, J., Yang, Y., Zimniak, P. & Awasthi, Y.C. (2003). Role of RLIP76 in lung cancer doxorubicin resistance: III. Anti-RLIP76 antibodies trigger apoptosis in lung cancer cells and synergistically increase doxorubicin cytotoxicity. International Journal of Oncology, Vol.22, No.4, (April 2003), pp. 721–732, ISSN 1019-6439

Awasthi, Y.C., Sharma, R., Sharma, A., Yadav, S., Singhal, S.S., Chaudhary, P. & Awasthi, S. (2008). Self-regulatory role of 4-hydroxynonenal in signaling for stress-induced programmed cell death. Free Radical Biology & Medicine, Vol.45, No.2, (July 2008), pp. 111-118, ISSN 0891-5849

Ayalasomayajula, S.P. & Kompella, U.B. (2002), Induction of vascular endothelial growth factor by 4-hydroxynonenal and its prevention by glutathione precursors in retinal pigment epithelial cells. European Journal of Pharmacology, Vol.449, No.3, (August 2002), pp. 213-220, ISSN 0014-2999

Baler, R., Dahl, G., & Voellmy, R. (1993). Activation of human heat shock genes is accompanied by oligomerization, modification, and rapid translocation of heat shock transcription factor HSF. Molecular and Cellular Biology, Vol.13, No.4, (April 1993), pp. 2486–2496, ISSN 0270-7306

Balogh, L.M. & Atkins, W.M. (2011). Interactions of glutathione transferases with 4-hydroxynonenal. Drug Metabolism Review, Vol.43, No.2, (May 2011), pp.165-178, ISSN 0360-2532

Barrera, G., Martinotti, S., Fazio, V., Manzari, V., Paradisi, L., Parola, M., Frati, L. & Dianzani, M.U. (1987). Effect of 4-hydroxynonenal on c-myc expression. Toxicologic Pathology, Vol.15, No.2, (1987), pp. 238-240, ISSN 0192-6233

Barrera, G., Di Mauro, C., Muraca, R., Ferrero, D., Cavalli, G., Fazio, V.M., Paradisi, L. & Dianzani, M.U. (1991). Induction of differentiation in human HL-60 cells by 4-hydroxynonenal.; a product of lipid peroxidation. Experimental Cell Research, Vol.197, No.2, (December 1991), pp. 148-152, ISSN 0014-4827

Bartel, D.P. (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. Cell, Vol.116, No.2, (January 2004), pp. 281–297, ISSN 0092-8674

Bartsch, H. & Nair, J. (2005). Accumulation of lipid peroxidation-derived DNA lesions: potential lead markers for chemoprevention of inflammation-driven malignancies. Mutation Research, Vol.591, No.1-2, (December 2005), pp. 34-44, ISSN 0027-5107

Berquin, I.M., Edwards, I.J. & Chen, Y.Q. (2008). Multi-targeted therapy of cancer by omega-3 fatty acids. Cancer Letters, Vol.269, No.2, (October 2008), pp.363-377, ISSN 0304-3835

Biasi, F., Tessitore, L., Zanetti, D., Cutrin, J.C., Zingaro, B., Chiarpotto, E., Zarkovic, N., Serviddio, G. & Poli G. (2002). Associated changes of lipid peroxidation and transforming growth factor beta1 levels in human colon cancer during tumour progression. Gut, Vol.50, No.3, (March 2002), pp.361-367, ISSN 0017-5749

Biasi, F., Vizio, B., Mascia, C., Gaia, E., Zarkovic, N., Chiarpotto, E., Leonarduzzi, G. & Poli G. (2006). c-Jun N-terminal kinase upregulation as a key event in the proapoptotic interaction between transforming growth factor-beta1 and 4-hydroxynonenal in colon mucosa. Free Radical Biology & Medicine, Vol.41, No.3, (August. 2006), pp.443-54, ISSN 0891-5849

Boon, P.J., Marinho, H.S., Oosting, R. & Mulder, G.J. (1999). Glutathione conjugation of 4-hydroxytrans-2,3-nonenal in the rat in vivo, the isolated perfused liver and erythrocytes. Toxicology and Applied Pharmacology, Vol.159, No.3, (September 1999), pp.214–223, ISSN 0041-008X

Breton, J., Gage, M.C., Hay, A.W., Keen, J.N., Wild, C.P., Donnellan C, Findlay, J.B. & Hardie, L.J. (2008). Proteomic screening of a cell line model of esophageal carcinogenesis identifies cathepsin D and aldo-keto reductase 1C2 and 1B10 dysregulation in Barrett's esophagus and esophageal adenocarcinoma. Journal of Proteome Research, Vol.7, No.5 (May 2008), pp.1953–1962, ISSN 1535-3893

Cajelli, E., Ferraris, A. & Brambilla, G. (1987). Mutagenicity of 4-hydroxynonenal in V79 Chinese hamster cells. Mutation Research, Vol.190, No.2, (February 1987), pp.169-171, ISSN 0027-5107

Calonghi, N., Boga, C., Cappadone, C., Pagnotta, E., Bertucci, C., Fiori, J. & Masotti, L. (2002). Cytotoxic and cytostatic effects induced by 4-hydroxynonenal in human osteosarcoma cells. Biochemical Biophysical Research Communcations, Vol.293, No.5, (May 2002), pp.1502-1507, ISSN 0006-291X

Canuto, R.A., Muzio, G., Ferro, M., Maggiora, M., Federa, R., Bassi, A.M., Lindahl, R. & Dianzani, M.U. (1999). Inhibition of class-3 aldehyde dehydrogenase and cell growth by restored lipid peroxidation in hepatoma cell lines. Free Radical Biology & Medicine, Vol.26, No.3-4, (February 1999), pp. 333-340, ISSN 0891-5849

Cassidy, A., Bingham, S. & Cummings, J. (1994). Starch intake and colorectal cancer risk: an international comparison. British Journal of Cancer, Vol.69, No.5, (May 1994), pp. 937-942, ISSN 0007-0920

Cerbone, A., Toaldo, C., Laurora, S., Briatore, F., Pizzimenti, S., Dianzani, M.U., Ferretti, C. & Barrera G. (2007). 4-Hydroxynonenal and PPARgamma ligands affect proliferation, differentiation, and apoptosis in colon cancer cells. Free Radical Biology & Medicine, Vol. 42, No.11, (June 2007), pp.1661-1670, ISSN 0891-5849

Chandramathi, S., Suresh, K., Anita, Z.B. & Kuppusamy, U.R. (2009). Comparative assessment of urinary oxidative indices in breast and colorectal cancer patients. Journal of Cancer Research and Clinical Oncology, Vol.135, No.2, (February 2009), pp.319–323, ISSN 0171-5216

Chen, J., Adikari, M., Pallai, R., Parekh, H.K. & Simpkins, H. (2008). Dihydrodiol dehydrogenases regulate the generation of reactive oxygen species and the development of cisplatin resistance in human ovarian carcinoma cells. Cancer Chemotherapy and Pharmacology, Vol.61, No.6, (May 2008), pp.979–987, ISSN 0344-5704

Cheng, J.Z., Singhal, S.S., Saini, M., Singhal, J., Piper, J.T., Van Kuijk, F.J., Zimniak, P., Awasthi, Y.C. & Awasthi, S. (1999). Effects of mGST A4 transfection on 4-hydroxynonenal-mediated apoptosis and differentiation of K562 human erythroleukemia cells. Archives of Biochemistry and Biophysics, Vol.372, No.1, (December 1999), pp. 29-36, ISSN 0003-9861

Cheng, J.Z., Sharma, R., Yang, Y., Singhal, S.S., Sharma, A., Saini, M.K., Singh, S.V., Zimniak, P., Awasthi, S. & Awasthi, Y,C. (2001). Accelerated metabolism and exclusion of 4-hydroxy-nonenal through induction of RLIP76 and hGST5.8 is an early adaptive response of cells to heat and oxidative stress. The Journal of Biological Chemistry, Vol.276, No.44, (November 2001), pp.41213–41223, ISSN 0021-9258

Chiarpotto, E., Scavazza, A., Leonarduzzi, G., Camandola, S., Biasi, F., Teggia, P.M., Garavoglia, M., Robecchi, A., Roncari, A. & Poli, G. (1997). Oxidative damage and transforming growth factor beta 1 expression in pretumoral and tumoral lesions of human intestine. Free Radical Biology & Medicine, Vol.22, No.5, (1997), pp.889-894, ISSN 0891-5849

Chung, F.L., Chen, H.J., Guttenplan, J.B., Nishikawa, A. & Hard, G.C. (1993). 2,3-epoxy-4-hydroxynonanal as a potential tumor-initiating agent of lipid peroxidation. Carcinogenesis, Vol.14, No.10, (October 1993), pp. 2073-2077, ISSN 0143-3334

Chung, F.L., Nath, R.G., Nagao, M., Nishikawa, A., Zhou, G.D. & Randerath, K. (1999). Endogenous formation and significance of 1,N2-propanodeoxyguanosine adducts. Mutation Research, Vol. 424, No.1-2, (March 1999), pp.71-78, ISSN 0027-5107

Chung, F.L., Nath, R.G., Ocando, J., Nishikawa, A. & Zhang, L. (2000). Deoxyguanosine adducts of t-4-hydroxy-2-nonenal are endogenous DNA lesions in rodents and humans: detection and potential sources. Cancer Research, Vol.60, No.6, (March 2000), pp.1507-1511, ISSN 0008-5472

Chung, F.L., Chen, H.J. & Nath, R.G. (1996). Lipid peroxidation as a potential endogenous source for the formation of exocyclic DNA adducts. Carcinogenesis, Vol.17, No.10, (October 1996), pp.:2105-2111, ISSN 0143-3334

Curzio M. (1988). Interaction between neutrophils and 4-hydroxyalkenals and consequences on neutrophil motility. Free Radical Research Communications, Vol.5, No.2, (1988), pp.55-66, ISSN 8755-0199

De Bont, R. & van Larebeke, N. (2004). Endogenous DNA damage in humans: a review of quantitative data. Mutagenesis, Vol.19, No.3, (May 2004), pp.169-185, ISSN 0267-8357

Desmots, F., Rissel, M., Loyer, P., Turlin, B. & Guillouzo, A. (2001). Immunohistological analysis of glutathione transferase A4 distribution in several human tissues using a specific polyclonal antibody. Journal of Histochemistry and Cytochemistry, Vol.49, No.12, (December 2001), pp.1573-1580, ISSN 0022-1554

Dianzani, M.U. (1993). Lipid peroxidation and cancer. Critical Reviews in Oncology/Hematology, Vol.15, No.2, (October 1993), pp.125-147, ISSN 1040-8428

Dianzani, M.U., Barrera, G. & Parola, M. (1999). 4-Hydroxy-2,3-nonenal as a signal for cell function and differentiation. Acta Biochimica Polonica, Vol.46, No.1, (1999), pp.61-75, ISSN 0001-527X

Dianzani, M.U. (2003). 4-hydroxynonenal from pathology to physiology. Molecular Aspects in Medicine, Vol.24, No.4-5, (August-October 2003), pp.263-272, ISSN 0098-2997

Douki, T., Odin, F., Caillat, S., Favier, A. & Cadet, J. (2004). Predominance of the 1,N2-propano 20-deoxyguanosine adduct among 4-hydroxy-2-nonenal-induced DNA lesions. Free Radical Biology & Medicine, Vol. 37, No.1, (July 2004), pp.62-70, ISSN 0891-5849

Ebert, M.N., Beyer-Sehlmeyer, G., Liegibel, U.M., Kautenburger, T., Becker, T.W. & Pool-Zobel B.L. (2001). Butyrate induces glutathione S-transferase in human colon cells and protects from genetic damage by 4-hydroxy-2-nonenal. Nutrition and Cancer, Vol.41, No.1-2, (2001), pp.156-164, ISSN 0163-5581

Eckl, P.M. (2003). Genotoxicity of HNE. Molecular Aspects in Medicine, Vol.24, No.4-5, (August-October 2003), pp.161-165, ISSN 0098-2997

Eder, E., Wacker, M., Lutz, U., Nair, J., Fang, X., Bartsch, H., Beland, F.A., Schlatter, J. & Lutz, W.K. (2006). Oxidative stress related DNA adducts in the liver of female rats fed with sunflower-, rapeseed-, olive- or coconut oil supplemented diets. Chemico-Biological Interactions, Vol.159, No.2, (February 2006), pp. 81-89, ISSN 0009-2797

Emerit, I., Khan, S.H. & Esterbauer H. (1991). Hydroxynonenal, a component of clastogenic factors? Free Radical Biology & Medicine, Vol.10, No.6, (1991), pp.371-377. ISSN 0891-5849

Englyst, H.N., Kingman, S.M. & Cummings, J.H. (1992). Classification and measurement of nutritionally important starch fractions. European Journal of Clinical Nutrition, Vol. Suppl 2:S, (October 1992), pp.33-50, ISSN 0954-3007

Esterbauer, H., Cheeseman, K.H., Dianzani, M.U., Poli, G. & Slater, T.F. (1982). Separation and characterization of the aldehydic products of lipid peroxidation stimulated by ADP-Fe2+ in rat liver microsomes. Biochemical Journal, Vol.208, No.1, (October 1982), pp.129-140, ISSN 0264-6021

Esterbauer, H., Schaur, R.J. & Zollner, H. (1991). Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radical Biology & Medicine, Vol.11, No.1, (1991), pp. 81-128, ISSN 0891-5849

Falletti, O. & Douki, T. (2008). Low glutathione level favors formation of DNA adducts to 4-hydroxy-2(E)-nonenal, a major lipid peroxidation product. Chemical Research in Toxicology, Vol.21, No.11, (November 2008), pp.2097-2105, ISSN 0893-228X

Favero, A., Parpinel, M. & Montella, M. (1999). Energy sources and risk of cancer of the breast and colon-rectum in Italy. Advances in Experimental Medicine and Biology, Vol.472, (1999), pp. 51-55, ISSN 0065-2598

Fazio, V.M., Barrera, G., Martinotti, S., Farace, M.G., Giglioni, B., Frati, L., Manzari, V. & Dianzani, M.U. (1992). 4-Hydroxynonenal, a product of cellular lipid peroxidation, which modulates c-myc and globin gene expression in K562 erythroleukemic cells. Cancer Research, Vol.52, No.18, (September 1992), pp.4866-4871. ISSN 0008-5472

Fearon, E.R. & Vogelstein, B. (1990). A genetic model for colorectal tumorigenesis. Cell, Vol.61, No.5, (June 1990), pp.759-767, ISSN 0092-8674

Feng, Z.H., Hu, W.W., Amin, S. & Tang, M.S. (2003). Mutational spectrum and genotoxicity of the major lipid peroxidation product, trans-4-hydroxy-2-nonenal, induced DNA adducts in nucleotide excision repairproficient and -deficient human cells. Biochemistry, Vol.42, No.25, (July 2003), pp.7848-7854, ISSN 0006-2960

Feng, Z., Hu, W. & Tang, M.S. (2004). Trans-4-hydroxy-2-nonenal inhibits nucleotide excision repair in human cells: a possible mechanism for lipid peroxidation-induced carcinogenesis. Proceedings of the National Academy of Sciences of the United States of America, Vol.101, No.23, (June 2004), pp.8598-8602, ISSN 0027-8424

Fernandes, P.H., Wang, H., Rizzo, C.J. & Lloyd, R.S. (2003). Site-specific mutagenicity of stereochemically defined 1,N-2-deoxyguanosine adducts of trans-4-hydroxynonenal in mammalian cells. Environmental and Molecular Mutagenesis, Vol.42, No.2, (2003), pp.68-74, ISSN 0893-6692

Forman, H.J., Fukuto, J.M., Miller, T., Zhang, H., Rinna, A. & Levy, S. (2008).The chemistry of cell signaling by reactive oxygen and nitrogen species and 4-hydroxynonenal. Archives of Biochemistry and Biophysics, Vol.477, No,2, (September 2008), pp.183-195, ISSN 0003-9861

Franceschi, S., La Vecchia, C., Russo, A., Favero, A., Negri, E., Conti, E., Montella, M., Filiberti, R., Amadori, D. & Decarli, A. (1998). Macronutrient intake and risk of colorectal cancer in Italy. International Journal of Cancer, Vol.76, No.3, (May 1998), pp.321-324, ISSN 0020-7136

Fukumoto, S., Yamauchi, N., Moriguchi, H., Hippo, Y., Watanabe, A., Shibahara, J., Taniguchi, H., Ishikawa, S., Ito, H., Yamamoto, S., Iwanari, H., Hironaka, M.,

Ishikawa, Y., Niki, T., Sohara, Y., Kodama, T., Nishimura, M., Fukayama, M., Dosaka-Akita, H. & Aburatani, H. (2005).
Overexpression of the aldo-keto reductase family protein AKR1B10 is highly correlated with smokers' non-small cell lung carcinomas. Clinical Cancer Research, Vol.11, No.5, (March 2005), pp.1776-1785, ISSN 1078-0432

Gasc, N., Taché, S., Rathahao, E., Bertrand-Michel, J., Roques, V. & Guéraud, F. (2007). 4-hydroxynonenal in foodstuffs: heme concentration, fatty acid composition and freeze-drying are determining factors. Redox Report, Vol.12, No.1, (2007), pp.40-44, ISSN 1351-0002

Gentile, F., Pizzimenti, S., Arcaro, A., Pettazzoni, P., Minelli, R., D'Angelo, D., Mamone, G., Ferranti, P., Toaldo, C., Cetrangolo, G., Formisano, S., Dianzani, M.U., Uchida, K., Dianzani, C. & Barrera, G. (2009). Exposure of HL-60 human leukaemic cells to 4-hydroxynonenal promotes the formation of adduct(s) with alpha-enolase devoid of plasminogen binding activity. Biochemical Journal, Vol.422, No.2, (August 2009), pp.285-294, ISSN 0264-6021

Glei, M., Hofmann, T., Küster, K., Hollmann, J., Lindhauer, M.G. & Pool-Zobel B.L. (2006) Both wheat (Triticum aestivum) bran arabinoxylans and gut flora-mediated fermentation products protect human colon cells from genotoxic activities of 4-hydroxynonenal and hydrogen peroxide. Journal of Agricultural and Food Chemistry, Vol.54, No.6, (March 2006), pp.2088-2095, ISSN 0021-8561

Glei, M., Schaeferhenrich, A., Claussen, U., Kuechler, A., Liehr, T., Weise, A., Marian, B., Sendt, W. & Pool-Zobel B.L. (2007). Comet fluorescence in situ hybridization analysis for oxidative stress-induced DNA damage in colon cancer relevant genes. Toxicological Sciences, Vol.96, No.2 (April 2007), pp. 279-284, ISSN 1096-6080

Glei, M., Hovhannisyan, G. & Pool-Zobel, B.L. (2009) Use of Comet-FISH in the study of DNA damage and repair: review. Mutation Research, Vol.681, No.1, (January-February 2009), pp.33-43, ISSN 0027-5107

Glinghammar, B., Venturi, M., Rowland, I.R. & Rafter, J.J. (1997). Shift from a dairy product-rich to a dairy product-free diet: influence on cytotoxicity and genotoxicity of fecal water—potential risk factors for colon cancer. American Journal of Clinical Nutrition, Vol.66, No.5, (November 1997), pp. 1277-1282, ISSN 0002-9165

Grune, T., Siems, W., Kowalewski, J., Zollner, H. & Esterbauer H. (1991). Identification of metabolic pathways of the lipid peroxidation product 4-hydroxynonenal by enterocytes of rat small intestine. Biochemical International, Vol.25, No.5, (December 1991), pp.963-971, ISSN 0158-5231

Gupta, A., Bhatt, M.L. & Misra, M.K. (2009). Lipid peroxidation and antioxidant status in head and neck squamous cell carcinoma patients. Oxidative Medicine and Cellular Longevity, Vol.2 , No.2, (April-June 2009), pp.68-72, ISSN 1942-0900

Hahn, G.M. & Li, G.C. (1982). Thermotolerance and heat shock proteins in mammalian cells. Radiation Research, Vol.92, No.3, (December 1982), pp.452-457, ISSN 0033-7587

Halliwell, B. (2007). Oxidative stress and cancer: have we moved forward? Biochemical Journal, Vol.401, No.1, (January 2007), pp.1-11, ISSN 0264-6021

Hartley, D.P, Ruth, J.A. & Petersen, D.R. (1995). The hepatocellular metabolism of 4-hydroxynonenal by alcohol dehydrogenase, aldehyde dehydrogenase, and

glutathione S-transferas,. Archives of Biochemistry and Biophysics, Vol.316, No.1, (January 1995), pp.197-205, ISSN 0003-9861

Harvey, C.J., Thimmulappa, R.K., Singh, A., Blake, D.J., Ling, G., Wakabayashi, N., Fujii, J., Myers, A. & Biswal, S. (2009). Nrf2-regulated glutathione recycling independent of biosynthesis is critical for cell survival during oxidative stress. Free Radical Biology and Medicine, Vol.46, No.4, (Febraury 2009), pp.443-453, ISSN 0891-5849

Hassane, D.C., Guzman, M.L., Corbett, C., Li, X., Abboud, R., Young, F., Liesveld, J.L.,Carroll, M. & Jordan, C.T. (2008). Discovery of agents that eradicate leukemia stem cells using an in silico screen of public gene expression data. Blood, Vol.111, No.12, (June 2008), pp.5654-5662, ISSN 0006-4971

Howard, M.K., Burke, L.C., Mailhos, C., Pizzey, A., Gilbert, C.S., Lawson, W.D., Collins, M.K., Thomas, N.S. & Latchman, D.S. (1993). Cell cycle arrest of proliferating neuronal cells by serum deprivation can result in either apoptosis or differentiation. Journal of Neurochemistry, Vol.60, No.5, (May 1993), pp.1783-1791, ISSN 0022-3042

Hsu, I.C., Metcalf, R.A., Sun, T., Welsh, J.A., Wang, N.J. & Harris, C.C. (1991). Mutational hotspot in the p53 gene in human hepatocellular carcinomas. Nature, Vol.350, No.6317, (April 1991), pp.427-428, ISSN 0028-0836

Hu, W., Feng, Z., Eveleigh, J., Lyer, G., Pan, J., Amin, S., Chung, F.T. & Tang, M.S. (2002). The major lipid peroxidation product, trans-4-hydroxy-2-nonenal, preferentially forms DNA adducts at codon 249 of human p53 gene, a unique mutational hotspot in hepatocellular carcinoma. Carcinogenesis, Vol.23, No.11, (November 2008), pp.1781-1789, ISSN 0143-3334

Huang, Y.L., Sheu, J.Y. & Lin, T.H. (1999). Association between oxidative stress and changes of trace elements in patients with breast cancer. Clinical Biochemistry, Vol.32, No.2, (March 1999), pp.131-136, ISSN 0009-9120

Hussain, S.P., Raja, K., Amstad, P.A., Sawyer, M., Trudel, L.J., Wogan, G.N., Hofseth, L.J., Shields, P.G., Billiar, T.R., Trautwein, C., Hohler, T., Galle, P.R., Phillips, D.H., Markin, R., Marrogi, A.J. & Harris, C.C. (2000). Increased p53 mutation load in nontumorous human liver of wilson disease and hemochromatosis: oxyradical overload diseases. Proceedings of the National Academy of Sciences of the United States of America, Vol.97, No.23, (November 2000), pp.12770-12775, ISSN 0027-8424

Isom, A.L., Barnes, S., Wilson, L., Kirk, M., Coward, L. & Darley-Usmar, V. (2004). Modification of Cytochrome c by 4-hydroxy- 2-nonenal: evidence for histidine, lysine, and arginine-aldehyde adducts. Journal of The American Society for Mass Spectrometry, Vol.15, No.8, (August 2004), pp.1136-1147, ISSN 1044-0305

Jacobs, A.T. & Marnett, L.J. (2007). Heat shock factor 1 attenuates 4-Hydroxynonenal-mediated apoptosis: critical role for heat shock protein 70 induction and stabilization of Bcl-XL. The Journal of Biological Chemistry, Vol.282, No.46, (November 2007), pp.33412-33420, ISSN 0021-9258

Jacobs, A.T. & Marnett, L.J. (2009). HSF1-mediated BAG3 expression attenuates apoptosis in 4-hydroxynonenal-treated colon cancer cells via stabilization of anti-apoptotic Bcl-2 proteins. The Journal of Biological Chemistry, Vol.284, No.14, (April 2009), pp.9176-9183, ISSN 0021-9258

Ji, C., Amarnath, V., Pietenpol, J.A. & Marnett, L.J. (2001a). 4-hydroxynonenal induces apoptosis via caspase-3 activation and cytochrome c release. Chemical Research in Toxicology, Vol.14, No.8, (August 2001), pp.1090-1096, ISSN 0893-228X

Ji, C., Kozak, K.R. & Marnett, L.J. (2001b). IkappaB kinase, a molecular target for inhibition by 4-hydroxy-2-nonenal. The Journal of Biological Chemistry, Vol.276, No.21, (May 2001), pp.18223-18228, ISSN 0021-9258

Kakishita, H. & Hattori, Y. (2001).Vascular smooth muscle cell activation and growth by 4-hydroxynonenal. Life Sciences, Vol.69, No.6, (June 2001), pp.689-97, ISSN 0024-3205

Karihtala, P., Kauppila, S., Puistola, U. & Jukkola-Vuorinen, A. (2011). Divergent behaviour of oxidative stress markers 8-hydroxydeoxyguanosine (8-OHdG) and 4-hydroxy-2-nonenal (HNE) in breast carcinogenesis. Histopathology, Vol.58, No.6, (May 2011), pp.854-862, ISSN 1365-2559

Kaspar, J.W. & Jaiswal, A.K. (2010).An autoregulatory loop between Nrf2 and Cul3-Rbx1 controlstheir cellular abundance. The Journal of Biological Chemistry, Vol.285, No.28, (July 2010), pp.21349-21358, ISSN 0021-9258

Katic, J., Cemeli, E., Baumgartner, A., Laubenthal, J., Bassano, I., Stølevik, S.B., Granum, B., Namork, E., Nygaard, U.C., Løvik, M., van Leeuwen, D., Vande Loock, K., Anderson, D., Fucić, A. & Decordier, I. (2010). Evaluation of the genotoxicity of 10 selected dietary/environmental compounds with the in vitro micronucleus cytokinesis-block assay in an interlaboratory comparison. Food and Chemical Toxicology, Vol.48, No.10, (June 2010), pp.2612-2623, ISSN 0278-6915

Kawamura, I., Lacey, E., Inami, M., Nishigaki, F., Naoe, Y., Tsujimoto, S., Manda, T. & Goto, T. (1999). Ponalrestat, an aldose reductase inhibitor, inhibits cachexia syndrome in nude mice bearing human melanomas G361 and SEKI. Anticancer Research, Vol.19, No.5B, (September-October 1999), pp.4091-4097, ISSN 0250-7005

Kim, Y.S. & Milner, J.A. (2007). Dietary modulation of colon cancer risk. Journal of Nutrition, Vol.137, No.11 Suppl, (November 2007), pp.2576S-2579S, ISSN 0022-3166

Kim, T.H., Hur, E.G., Kang, S.J., Kim, J.A., Thapa, D., Lee, Y.M., Ku, S.K., Jung, Y. & Kwak, M.K. (2011). NRF2 blockade suppresses colon tumor angiogenesis by inhibiting hypoxia-induced activation of HIF-1α. Cancer Research, Vol.71, No.6, (March 2011), pp.2260-2275, ISSN 0008-5472

Knoll, N., Ruhe, C., Veeriah, S., Sauer, J., Glei, M., Gallagher, E.P. & Pool-Zobel, B.L. (2005). Genotoxicity of 4-hydroxy-2-nonenal in human colon tumor cells is associated with cellular levels of glutathione and the modulation of glutathione S-transferase A4 expression by butyrate. Toxicological Sciences, Vol.86, No.1, (July 2005), pp.27-35, ISSN 1096-6080

Kondo, S., Toyokuni, S., Iwasa, Y., Tanaka, T., Onodera, H., Hiai, H. & Imamura, M. (1999). Persistent oxidative stress in human colorectal carcinoma, but not in adenoma. Free Radical Biology and Medicine, Vol.27, No.3-4, (August 1999), pp.401-410, ISSN 0891-5849

Konner, J., O'Reilly, E. (2002). Pancreatic cancer; epidemiology, genetics, and approaches to screening. Oncology (Williston Park, N.Y.), Vol.16, No.12, (December 2002), pp.1631-1638, ISSN 0890-9091

Koster, J.F., Slee, R.G., Montfoort, A., Lang, J. & Esterbauer, H. (1986). Comparison of the inactivation of microsomal glucose-6-phosphatase by in situ lipid peroxidation-derived 4-hydroxynonenal and exogenous 4-hydroxynonenal. Free radical research communications, Vol.1, No.4, (1986), pp.273-287, ISSN 8755-0199

Kotrikadze, N., Alibegashvili, M., Zibzibadze, M., Abashidze, N., Chigogidze, T., Managadze, L. & Artsivadze, K. (2008). Activity and content of antioxidant enzymes in prostate tumors. Experimental Oncology, Vol.30, No.3, (September 2008), pp.244-247, ISSN 1812-9269

Kowalczyk, P., Ciesla, J.M., Komisarski, M., Kusmierek, J.T. & Tudek, B. (2004). Long-chain adducts of trans-4-hydroxy-2-nonenal to DNA bases cause recombination, base substitutions and frameshift mutations in M13 phage. Mutation Research, Vol.550, No.1-2, (June 2004), pp.33-48, ISSN 1383-5718

Lapre, J.A. & Vandermeer, R. (1992) Diet-induced increase of colonic bile acids stimulates lytic activity of fecal water and proliferation of colonic cells. Carcinogenesis, Vol.13, No.1, (January 1992), pp.41-44, ISSN 0143-3334

Laurent, A., Alary, J., Debrauwer, L. & Cravedi, J.P. (1999). Analysis in the rat of 4-hydroxynonenal metabolites excreted in bile: Evidence of enterohepatic circulation of these byproducts of lipid peroxidation. Chemical Research in Toxicology, Vol.12, No.10, (October 1999), pp.887-894, ISSN 0893-228X

Laurora, S., Tamagno, E., Briatore, F., Bardini, P., Pizzimenti, S., Toaldo, C., Reffo, P., Costelli, P., Dianzani, M.U., Danni, O. & Barrera, G. (2005). 4-Hydroxynonenal modulation of p53 family gene expression in the SK-N-BE neuroblastoma cell line. Free Radical Biology and Medicine, Vol.38, No.2, (January 2005), pp.215-25, ISSN 0891-5849

Leonarduzzi, G., Robbesyn, F. & Poli, G. Signaling kinases modulated by 4-hydroxynonenal. (2004). Free Radical Biology and Medicine, Vol.37, No.11, (December 2004), pp.1694-1702, ISSN 0891-5849

Lin, M.Y. & Yen, C.L. (1999). Antioxidative ability of lactic acid Bacteria. Journal of Agricultural and Food Chemistry, Vol.47, No.4, (April 1999), pp.1460-1466, ISSN 0021-8561

Looi, M.L., Mohd Dali, A.Z., Md Ali, S.A., Wan Ngah, W.Z. & Mohd Yusof, Y.A. (2008). Oxidative damage and antioxidant status in patients with cervical intraepithelial neoplasia and carcinoma of the cervix. European Journal of Cancer Prevention, Vol.17, No.6, (November 2008), pp.555-560, ISSN 0959-8278

Malhotra, D., Portales-Casamar, E., Singh, A., Srivastava, S., Arenillas, D., Happel, C., Shyr, C., Wakabayashi, N., Kensler, T.W., Wasserman, W.W. & Biswal, S. (2010). Global mapping of 22 binding sites for Nrf2 identifies novel targets in cell survival response through ChIP-Seq profiling and network analysis. Nucleic Acids Research, Vol.38, No.17, (September 2010), pp.5718-5734, ISSN 0305-1048

Martin, H.J. & Maser, E. (2009). Role of human aldo-keto-reductase AKR1B10 in the protection against toxic aldehydes. Chemico-Biological Interactions, Vol.178, No.1-3, (March 2009), pp.145-150, ISSN 0009-2797

Matsunaga, T., Yamane, Y., Iida, K., Endo, S., Banno, Y., El-Kabbani, O. & Hara, A. (2011). Involvement of the aldo-keto reductase, AKR1B10, in mitomycin-c resistance

through reactive oxygen species-dependent mechanisms. Anti-Cancer Drugs, Vol.22, No.5, (June 2011), pp.402-408, ISSN 1473-5741

Min, K. & Ebeler, S.E. (2009).Quercetin inhibits hydrogen peroxide-induced DNA damage and enhances DNA repair in Caco-2 cells. Food and Chemical Toxicology. Vol.47, No.11, (November 2009), pp.2716-2722, ISSN 0278-6915

Moseley, P.L. (1997). Heat shock proteins and heat adaptation of the whole organism. Journal of Applied Physiology, Vol.83, No.5, (November 1997), pp.1413-1417, ISSN 8750-7587

Murawaki, Y., Tsuchiya, H., Kanbe, T., Harada, K., Yashima, K., Nozaka, K., Tanida, O., Kohno, M., Mukoyama, T., Nishimuki, E., Kojo, H., Matsura, T., Takahashi, K., Osaki, M., Ito, H., Yodoi, J., Murawaki, Y. & Shiota, G. (2008). Aberrant expression of selenoproteins in the progression of colorectal cancer. Cancer Letters, Vol.259, No.2, (Febraury 2008), pp.218-230, ISSN 0304-3835

Muzio, G., Canuto, R.A., Trombetta, A. & Maggiora, M. (2001). Inhibition of cytosolic class 3 aldehyde dehydrogenase by antisense oligonucleotides in rat hepatoma cells. Chemico-Biological Interactions, Vol.130-132, No.1-3, (January 2001), pp.219-225, ISSN 0009-2797

Nair, J., Gansauge, F., Beger, H., Dolara, P., Winde, G. & Bartsch, H. (2006). Increased etheno-DNA adducts in affected tissues of patients suffering from Crohn's disease, ulcerative colitis, and chronic pancreatitis. Antioxidants & Redox Signaling, Vol.8, No.5-6, (May-June 2006), pp.1003-1010, ISSN 1523-0864

Nishikawa, A., Furukawa, F., Kasahara, K., Ikezaki, S., Itoh, T., Suzuki, T., Uchida, K., Kurihara, M., Hayashi, M., Miyata, N. & Hirose, M. (2000). Trans-4-hydroxy-2-nonenal, an aldehydic lipid peroxidation product, lacks genotoxicity in lacI transgenic mice. Cancer Letters, Vol.148, No.1, (January 2000), pp.81-86, ISSN 0304-3835

Obtułowicz, T., Winczura, A., Speina, E., Swoboda, M., Janik, J., Janowska, B., Cieśla, J.M., Kowalczyk, P., Jawien, A., Gackowski, D., Banaszkiewicz, Z., Krasnodebski, I., Chaber, A., Olinski, R., Nair, J., Bartsch, H., Douki, T., Cadet, J. & Tudek, B. (2010). Aberrant repair of etheno-DNA adducts in leukocytes and colon tissue of colon cancer patients. Free Radical Biology and Medicine, Vol.49, No.6, (September 2010), pp.1064-1071, ISSN 0891-5849

Okamoto, K., Toyokuni, S., Uchida, K., Ogawa, O., Takenawa, J., Kakehi, Y., Kinoshita, H., Hattori-Nakakuki, Y., Hiai, H. & Yoshida, O. (1994). Formation of 8-hydroxy-2'-deoxyguanosine and 4-hydroxy-2-nonenal-modified proteins in human renal-cell carcinoma. International Journal of Cancer, Vol.58, No.6, (September 1994), pp.825-829, ISSN 1097-0215

Pan, J.S., Hong, M.Z. & Ren, J.L. (2009). Reactive oxygen species: a double-edged sword in oncogenesis. World Journal of Gastroenterology, Vol.15, No.14, (April 2009), pp.1702-1707, ISSN 1007-9327

Park, J.H. & Park, E. (2011). Influence of iron-overload on DNA damage and its repair in human leukocytes in vitro. Mutation Research, Vol.718, No.1-2, (January 2011), pp.56-61, ISSN 1383-5718

Parola, M., Bellomo, G., Robino, G., Barrera, G. & Dianzani, M.U. (1999). 4-Hydroxynonenal as a biological signal: molecular basis and pathophysiological implications.

Antioxidants & Redox Signaling, Vol.3, No.1, (Fall 1999), pp.255-284, ISSN 1523-0864

Pearson, J.R., Gill, C.I. & Rowland, I.R. (2004). Diet, fecal water, and colon cancer--development of a biomarker. Nutrition Reviews, Vol.67, No.9, (September 2009), pp.509-526, ISSN 0029-6643

Petersen, D.R. & Doorn, J.A. (2004). Reactions of 4-hydroxynonenal with proteins and cellular targets. Free Radical Biology and Medicine, Vol.37, No.7, (October 2004), pp.937-945, ISSN 0891-5849

Pettazzoni, P., Pizzimenti, S., Toaldo, C., Sotomayor, P., Tagliavacca, L., Liu, S., Wang, D., Minelli, R., Ellis, L., Atadja, P., Ciamporcero, E., Dianzani, M.U., Barrera, G. & Pili, R. (2011). Induction of cell cycle arrest and DNA damage by the HDAC inhibitor panobinostat (LBH589) and the lipid peroxidation end product 4-hydroxynonenal in prostate cancer cells. Free Radical Biology and Medicine, Vol.50, No.2, (January 2011), pp.313-322, ISSN 0891-5849

Pierre, F., Tache, S., Petit, C.R., Van der Meer, R. & Corpet, D.E. (2003) Meat and cancer: haemoglobin and haemin in a low-calcium diet promote colorectal carcinogenesis at the aberrant crypt stage in rats. Carcinogenesis, Vol.24, No.10, (October 2003), pp.1683-1690, ISSN 0143-3334

Pierre, F., Freeman, A., Tache, S., Van der Meer, R. & Corpet, D.E. (2004). Beef meat and blood sausage promote the formation of azoxymethaneinduced mucin-depleted foci and aberrant crypt foci in rat colons. Journal of Nutrition, Vol.134, No.10, (October 2004), pp.2711-2716, ISSN 0022-3166

Pierre, F., Peiro, G., Tache, S., Cross, A.J., Bingham, S.A., Gasc, N., Gottardi, G., Corpet, D.E. & Guéraud, F. (2006). Newmarker of colon cancer risk associated with heme intake: 1,4-dihydroxynonane mercapturic acid. Cancer Epidemiology, Biomarkers & Prevention, Vol.15, No.11, (November 2006), pp.2274-2279, ISSN 1538-7755

Pierre, F., Tache, S., Guéraud, F., Rerole, A.L., Jourdan, M.L. & Petit, C. (2007). Apc mutation induces resistance of colonic cells to lipoperoxide-triggered apoptosis induced by faecal water from haem-fed rats. Carcinogenesis, Vol.28, No.2, (February 2007), pp.321-327, ISSN 0143-3334

Pizzimenti, S., Briatore, F., Laurora, S., Toaldo, C., Maggio, M., De Grandi, M., Meaglia, L., Menegatti, E., Giglioni, B., Dianzani, M.U. & Barrera, G. (2006). 4-Hydroxynonenal inhibits telomerase activity and hTERT expression in human leukemic cell lines. Free Radical Biology and Medicine, Vol.40, No.9, (May 2006), pp.1578-1591, ISSN 0891-5849

Pizzimenti, S., Ferracin, M., Sabbioni, S., Toaldo, C., Pettazzoni, P., Dianzani, M.U., Negrini, M. & Barrera, G. (2009). MicroRNA expression changes during human leukemic HL-60 cell differentiation induced by 4-hydroxynonenal, a product of lipid peroxidation. Free Radical Biology and Medicine, Vol.46, No.2, (January 2009), pp.282-288, ISSN 0891-5849

Pizzimenti, S., Toaldo, C., Pettazzoni, P., Dianzani, M.U. & Barrera, G. (2010a). The "Two-Faced" Effects of Reactive Oxygen Species and the Lipid Peroxidation Product 4-Hydroxynonenal in the Hallmarks of Cancer. Cancers, Vol.2, No.2, (March 2010), pp.338-363, ISSN 1097-0142

Pizzimenti, S., Menegatti, E., Berardi, D., Toaldo, C., Pettazzoni, P., Minelli, R., Giglioni, B., Cerbone, A., Dianzani, M.U., Ferretti, C. & Barrera, G. (2010b). 4-hydroxynonenal, a lipid peroxidation product of dietary polyunsaturated fatty acids, has anticarcinogenic properties in colon carcinoma cell lines through the inhibition of telomerase activity. The Journal of Nutritional Biochemistry, Vol.21, No.9, (September 2010), pp.818-826, ISSN 0955-2863

Poli, G., Schaur, R.J., Siems, W.G. & Leonarduzzi, G. (2008). 4-hydroxynonenal: a membrane lipid oxidation product of medicinal interest. Medicinal Research Reviews, Vol.28, No.4, (July 2008), pp.569-631, ISSN 0198-6325

Poljak-Blazi, M., Kralj, M., Hadzija, M.P., Zarković, N., Zarković, K. & Waeg, G. (2000). Involvement of lipid peroxidation, oncogene expression and induction of apoptosis in the antitumorous activity of ferric-sorbitol-citrate. Cancer Biotherapy and Radiopharmaceuticals, Vol.15, No.3, (June 2000), pp.285-293, ISSN 1084-9785

Ramana, K.V., Tammali, R. & Srivastava, S.K. (2010). Inhibition of aldose reductase prevents growth factor-induced G1-S phase transition through the AKT/phosphoinositide 3-kinase/E2F-1 pathway in human colon cancer cells. Molecular Cancer Therapeutics, Vol.9, No.4, (April 2010), pp.813-824, ISSN 1535-7163

Reddy, B.S. (2002). Types and amount of dietary fat and colon cancer risk: Prevention by omega-3 fatty acid-rich diets. Environmental Health and Preventive Medicine, Vol.7, No.3, (July 2002), pp.95-102, ISSN 1342-078X

Reddy, N.M., Kleeberger, S.R., Yamamoto, M., Kensler, T.W., Scollick, C., Biswal, S. & Reddy, S.P. (2007). Genetic dissection of the Nrf2-dependent redox signaling-regulated transcriptional programs of cell proliferation and cytoprotection. Physiological Genomics, Vol.32, No.1, (December 2007), pp. 74-81, ISSN 1094-8341

Richter, M., Jurek, D., Wrba, F., Kaserer, K., Wurzer, G., Karner-Hanusch, J. & Marian B. (2002). Cells obtained from colorectal microadenomas mirror early premalignant growth patterns in vitro. European Journal of Cancer, Vol.38, No.14, (September 2002), pp. 1937–1945. ISSN 0959-8049

Rinaldi, M., Barrera, G., Aquino, A., Spinsanti, P., Pizzimenti, S., Farace, M.G., Dianzani, M.U. & Fazio, V.M. (2000). 4-Hydroxynonenal-induced MEL cell differentiation involves PKC activity translocation. Biochemical and Biophysical Research Communications, Vol.272, No.1, (May 2000), pp. 75-80, ISSN 0006-291X

Sarge, K.D., Murphy, S.P., & Morimoto, R.I. (1993). Activation of heat shock gene transcription by heat shock factor 1 involves oligomerization, acquisition of DNA-binding activity, and nuclear localization and can occur in the absence of stress. Molecular and Cellular Biology, Vol.13, No.3, (March 1993), pp. 1392–1407, ISSN 0270-7306

Satow, R., Shitashige, M., Kanai, Y., Takeshita, F., Ojima, H., Jigami, T., Honda, K., Kosuge, T., Ochiya, T., Hirohashi, S. & Yamada T. (2010). Combined functional genome survey of therapeutic targets for hepatocellular carcinoma. Clinical Cancer Research, Vol.16, No.9, (May 2010), pp. 2518–2528, ISSN 1078-0432

Saw, C.L. & Kong, A.N. (2011). Nuclear factor-erythroid 2-related factor 2 as a chemopreventive target in colorectal cancer. Expert Opinion on Therapeutic Targets, Vol.15, No.3, (March 2011), pp. 281-295, ISSN 1472-8222

Sawa,T., Akaike,T., Kida, K., Fukushima,Y., Takagi, K., & Maeda, H. (1998). Lipid peroxyl radicals from oxidized oils and heme-iron: implication of a high-fat diet in colon carcinogenesis. Cancer Epidemiology, Biomarkers & Prevention, Vol.7, No.11, (November 1998), pp. 1007–1012, ISSN 1055-9965

Sayre, L.M., Arora, P.K., Iyer, R.S. & Salomon, R.G. (1993). Pyrrole formation from 4-hydroxynonenal and primary amines. Chemical Research in Toxicology , Vol.6, No.1, (January-February 1993), pp. 19-22, ISSN 0893-228X

Schaeferhenrich, A., Beyer-Sehlmeyer, G., Festag, G., Kuechler, A., Haag, N., Weise, A., Liehr, T., Claussen, U., Marian, B., Sendt, W., Scheele, J. & Pool-Zobel B.L. (2003). Human adenoma cells are highly susceptible to the genotoxic action of 4-hydroxy-2-nonenal. Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis, Vol.526, No.1-2, (May 2003), pp. 19-32, ISSN: 0027-5107

Scharlau, D., Borowicki, A., Habermann, N., Hofmann, T., Klenow, S., Miene, C., Munjal, U., Stein, K. & Glei M. (2009). Mechanisms of primary cancer prevention by butyrate and other products formed during gut flora-mediated fermentation of dietary fibre. Mutatation Research - Reviews, Vol.682, No.1, (July-August 2009), pp. 39-53, ISSN 1383-5742

Scharmach, E., Hessel, S., Niemann, B. & Lampen, A. (2009). Glutathione S-transferase expression and isoenzyme composition during cell differentiation of Caco-2 cells. Toxicology, Vol.265, No.3, (November 2009), pp. 122-126, ISSN 0300-483X

Schaur, R.J. (2003). Basic aspects of the biochemical reactivity of 4-hydroxynonenal. Molecular Aspects of Medicine, Vol.24, No. 4-5, (August-October 2003), pp. 149-59, ISSN: 0098-2997

Scheppach, W., Bingham, S., Boutron-Ruault, M., Verdier, M. G. D., Moreno, V., Nagengast, F., Reifen, R., Riboli, E., Seitz, H. &Wahrendorf, J. (1999). WHO consensus statement on the role of nutrition in colorectal cancer. European Journal of Cancer Prevention, Vol.8, No.1, (February 1999), pp. 57–62, ISSN 0959-8278

Schmid, K., Nair, J., Winde, G., Velic, I., & Bartsch, H. (2000). Increased levels of promutagenic etheno-DNA adducts in colonic polyps of FAP patients. International Journal of Cancer , Vol.87, No.1, (July 2000), pp. 1-4, ISSN 0020-7136

Seitz, H.K., Egerer, G., Oneta, C., Krämer, S., Sieg, A., Klee, F. & Simanowski, U.A. (1996). Alcohol dehydrogenase in the human colon and rectum. Digestion, Vol.57, No.2, pp. 105-108, ISSN 0012-2823

Selga, E., Noé, V., & Ciudad, C.J. (2008). Transcriptional regulation of aldo-keto reductase 1C1 in HT29 human colon cancer cells resistant to methotrexate: role in the cell cycle and apoptosis. Biochemical Pharmacology,. Vol.75, No.2, (January 2008), pp. 414–426, ISSN 0006-2952

Semlitsch, T., Tillian, H.M., Zarkovic, N., Borovic, S., Purtscher, M., Hohenwarter, O., & Schaur, R.J. (2002). Differential influence of the lipid peroxidation product 4-hydroxynonenal on the growth of human lymphatic leukaemia cells and human peripheral blood lymphocytes. Anticancer Research, Vol.22, No.3, (May-June 2002), pp. 1689-1697, ISSN 0250-7005

Sesink, A.L.A., Termont, D.S.M.L., Kleibeuker, J.H. & Vandermeer, R. (1999). Red meat and colon cancer: the cytotoxic and hyperproliferative effects of dietary heme. Cancer Research, Vol.59, No.22, (November 1999), pp. 5704–5709, ISSN 0008-5472

Sharma, R., Sharma, A., Yang, Y., Awasthi, S., Singhal, S.S., Zimniak, P. & Awasthi, Y.C. (2002). Functional reconstitution of Ral-binding GTPase activating protein, RLIP76, in proteoliposomes catalyzing ATP-dependent transport of glutathione conjugate of 4-hydroxynonenal. Acta Biochimica Polonica, Vol.49, No.3, pp. 693-701, ISSN 0001-527X

Siems, W. & Grune, T. (2003). Intracellular metabolism of 4-hydroxynonenal. Molecular Aspects of Medicine, Vol.24, No.4-5, (August-October 2003), pp. 167– 175, ISSN: 0098-2997

Singhal, S.S., Singhal, J., Yadav, S., Dwivedi, S., Boor, P.J., Awasthi, Y.C., & Awasthi, S. (2007). Regression of lung and colon cancer xenografts by depleting or inhibiting RLIP76 (Ral-binding protein 1). Cancer Research, Vol.67, No. 9, (May 2007), pp. 4382-4389, ISSN 0008-5472

Skrzydlewska, E., Sulkowski, S., Koda, M., Zalewski, B., Kanczuga-Koda, L. & Sulkowska, M. (2005). Lipid peroxidation and antioxidant status in colorectal cancer. World Journal of Gastroenterology, Vol.11, No.3, pp. 403-406, ISSN 1007-9327

Soong, R., Powell, B., Elsaleh, H., Gnanasampanthan, G., Smith, D.R., Goh, H.S., Joseph, D. & Iacopetta, B. (2000). Prognostic significance of TP53 gene mutation in 995 cases of colorectal carcinoma. Influence of tumour site, stage, adjuvant chemotherapy and type of mutation. European Journal of Cancer, Vol. 36, No.16, (October 2000), pp. 2053–2060, ISSN 0959-8049

Soussi, T., Dehouche, K. & Beroud, C. (2000). p53 website and analysis of p53 gene mutations in human cancer: forging a link between epidemiology and carcinogenesis. Human Mutation, Vol.15, No.1, pp. 105–113, ISSN: 1059-7794

Soussi, T. & Beroud, C. (2003). Significance of p53 mutations in human cancer: a critical analysis of mutations at CpG dinucleotides. Human Mutation, Vol.21, No3, pp. 192–200, ISSN: 1059-7794

Stagos, D., Zhou, H., Ross, D. & Vasiliou, V. (2009). 4-HNE inhibits tube formation and up-regulates chondromodulin-I in human endothelial cells. Biochemical and Biophysical Research Communications, Vol.379, No.3, (February 2009), pp. 654-658, ISSN 0006-291X

Sunjic, S.B., Cipak, A., Rabuzin, F., Wildburger, R. & Zarkovic, N. (2005). The influence of 4-hydroxy-2-nonenal on proliferation, differentiation and apoptosis of human osteosarcoma cells. Biofactors, Vol. 24, No.1-4, pp. 141-148, ISSN 1872-8081

Surh, J., & Kwon, H. (2005). Estimation of daily exposure to 4-hydroxy-2-alkenals in Korean foods containing n-3 and n-6 polyunsaturated fatty acids. Food Additives & Contaminants., Vol.22, No.8, (August 2005), pp. 701–708, ISSN 1944-0049

Surh, J., Lee, S. & Kwon, H. (2007). 4-Hydroxy-2-alkenals in polyunsaturated fatty acids-fortified infant formulas and other commercial food products. Food Additives & Contaminants, Vol. 24, No.11, (November 2007), pp. 1209-1218, ISSN 1944-0049

Surh, J., Lee, B.Y. & Kwon, H. (2010). Influence of Fatty Acids Compositions and Manufacturing Type on the Formation of 4-Hydroxy-2-alkenals in Food Lipids. Food Science and Biotechnology, Vol.19, No.2, (April 2010), pp.297-303, ISSN 1226-7708

Tammali, R., Ramana, K.V., Singhal, S.S., Awasthi, S., & Srivastava, S.K. (2006). Aldose reductase regulates growth factor-induced cyclooxygenase-2 expression and

prostaglandin E2 production in human colon cancer cells. Cancer Research, Vol.66, No.19, (October 2006), pp. 9705-9713, ISSN 0008-5472

Tammali, R., Srivastava, S.K. & Ramana, K.V. (2011). Targeting aldose reductase for the treatment of cancer. Current Cancer Drug Targets, Vol.11, No.5, (June 2011), pp. 560-571, ISSN 1568-0096

Thimmulappa, R.K., Mai, K.H., Srisuma, S., Kensler, T.W., Yamamoto, M. & Biswal, S. (2002). Identification of Nrf2-regulated genes induced by the chemopreventive agent sulforaphane by oligonucleotide microarray. Cancer Research, Vol.62, No.18, (September 2002), pp. 5196-5203, ISSN 0008-5472

Tice, R.P., Andrews, P.W., Hirai, O. & Singh, N.P. (1991). The single cell gel (SCG) assay: an electrophoretic technique for the detection of DNA damage in individual cells. Advances in Experimental Medicine and Biology, Vol. 283, pp. 157–164, ISSN 0065-2598

Uchida, K. (2003). 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress. Progress in lipid research, Vol.42, No.4, (July 2003), pp. 318-343, ISSN 0163-7827

Vander Jagt, D.L., Kolb, N.S., Vander Jagt, T.J., Chino, J., Martinez, F.J., Hunsaker, L.A. & Royer, R.E. (1995). Substrate specificity of human aldose reductase: Identification of 4-hydroxynonenal as an endogenous substrate. Biochimica et Biophysica Acta, Vol.1249, No.2, (June 1995), pp. 117–126, ISSN 0006-3002

Vatsyayan, R., Lelsani, P.C., Awasthi, S., & Singhal, S.S. (2010). RLIP76: a versatile transporter and an emerging target for cancer therapy. Biochemical Pharmacology, Vol.79, No.12, (June 2010), pp. 1699-1705, ISSN 0006-2952

Veitch, Z.W., Guo, B., Hembruff, S.L., Bewick, A.J., Heibein, A.D., Eng, J., Cull, S., Maclean, D.A., & Parissenti, A.M. (2009). Induction of 1C aldoketoreductases and other drug dose-dependent genes upon acquisition of anthracycline resistance. Pharmacogenetics and Genomics, Vol.19, No.6, (June 2009), pp. 477–488, ISSN 1744-6872

Vizio, B., Poli, G., Chiarpotto, E. & Biasi, F. (2005). 4-hydroxynonenal and TGF-beta1 concur in inducing antiproliferative effects on the CaCo-2 human colon adenocarcinoma cell line. Biofactors., Vol.24, No.1-4, pp. 237-246, ISSN 1872-8081

Vogelstein, B., Fearon, E.R., Hamilton, S.R., Kern, S.E., Preisinger, A.C., Leppert, M., Nakamura, Y., White, R., Smits, A.M. & Bos, J.L. (1988). Genetic alterations during colorectal tumor development. The New England Journal of Medicine, Vol.319, No.9, (September 1988), pp. 525–532, ISSN 0028-4793

Wacker, M., Wanek, P., Eder, E., Hylla, S., Gostner, A. & Scheppach, W. (2002). Effect of enzyme-resistant starch on formation of 1,N(2)-propanodeoxyguanosine adducts of trans-4-hydroxy-2-nonenal and cell proliferation in the colonic mucosa of healthy volunteers. Cancer Epidemiology, Biomarkers & Prevention, Vol.11, No.9, (September 2002), pp. 915-920, ISSN 1055-9965

Wacker, M., Wanek, P. & Eder, E. (2001). Detection of 1,N2-propanodeoxyguanosine adducts of trans-4-hydroxy-2-nonenal after gavage of trans-4-hydroxy-2-nonenal or induction of lipid peroxidation with carbon tetrachloride in F344 rats. Chemico-Biological Interactions, Vol.137, No.3, (September 2001), pp. 269–283, ISSN 0009-2797

Wang, J. & Yi, J. (2008). Cancer cell killing via ROS: to increase or decrease, that is the question. Cancer Biology and Therapy, Vol.7, No.12, (December 2008), pp. 1875-1884, ISSN 1538-4047

West, J.D., Ji, C., Duncan, S.T., Amarnath, V., Schneider, C., Rizzo, C.J., Brash, A.R. & Marnett L.J. (2004). Induction of apoptosis in colorectal carcinoma cells treated with 4-hydroxy-2-nonenal and structurally related aldehydic products of lipid peroxidation. Chemical Research in Toxicology, Vol. 17, No. 4, (April 2004), pp. 453-462, ISSN 0893-228X

West, J.D. & Marnett, L.J. (2005). Alterations in gene expression induced by the lipid peroxidation product, 4-hydroxy-2-nonenal. Chemical Research in Toxicology, Vol.18, No.11, (November 2001), pp. 1642-1653, ISSN 0893-228X

Winter, C.K., Segall. H.J. & Haddon.W.F. (1986). Formation of cyclic adducts of deoxyguanosine with the aldehydes trans-4-hydroxy-2-hexenal and trans-4-hydroxy-2-nonenal in vitro. Cancer Research, Vol.46, No.11, (November 1986), pp. 5682-5686, ISSN 0008-5472

Yadav, U.C., Ramana, K.V., Awasthi, Y.C. & Srivastava, S.K. (2008). Glutathione level regulates HNE-induced genotoxicity in human erythroleukemia cells. Toxicology and Applied Pharmacology, Vol.227, No.2, (March 2008), pp. 257-264, ISSN 0041-008X

Yan, R., Zu, X., Ma, J., Liu, Z., Adeyanju, M. & Cao, D. (2007). Aldo-keto reductase family 1B10 gene silencing results in growth inhibition of colorectal cancer cells: implication for cancer intervention. International Journal of Cancer, Vol.121, No.10, (November 2007), pp. 2301–2306, ISSN 0020-7136

Yang, Y., Sharma, A., Sharma, R., Patrick, B., Singhal, S.S., Zimniak, P., Awasthi, S. & Awasthi, Y.C. (2003). Cells preconditioned with mild, transient UVA irradiation acquire resistance to oxidative stress and UVA-induced apoptosis: role of 4-hydroxynonenal in UVA mediated signalling for apoptosis. The Journal of Biological Chemistry, Vol.278, No.42, (October 2003), pp. 41380–41388, ISSN 0021-9258

Yin, S.J., Liao, C.S., Lee, Y.C., Wu, C.W. & Jao, S.W. (1994).Genetic polymorphism and activities of human colon alcohol and aldehyde dehydrogenases: no gender and age differences. Alcoholism, clinical and experimental research, Vol.18, No.5, (October1994), pp. 1256-1260, ISSN 1530-0277

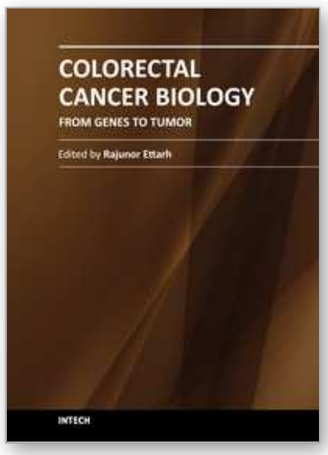
Yoshitake, H., Takahashi, M., Ishikawa, H., Nojima, M., Iwanari, H., Watanabe, A., Aburatani, H., Yoshida, K., Ishi, K., Takamori, K., Ogawa, H., Hamakubo, T., Kodama, T. & Araki, Y. (2007). Aldo-keto reductase family 1, member B10 in uterine carcinomas: a potential risk factor of recurrence after surgical therapy in cervical cancer. International Journal of Gynecological Cancer. Vol.17, No.6, (November-December 2007), pp. 1300–1306, ISSN 1048-891X

Zanetti, D., Poli, G., Vizio, B., Zingaro, B., Chiarpotto, E. & Biasi, F. (2003). 4-hydroxynonenal and transforming growth factor-beta1 expression in colon cancer. Molecular Aspects of Medicine, Vol.24, No.4-5, (August-October 2003), pp. 273-280, ISSN 0098-2997

Zarkovic, K., Juric, G., Waeg, G., Kolenc, D. & Zarkovic, N. (2005). Immunohistochemical appearance of HNE-protein conjugates in human astrocytomas. Biofactors, Vol.24, No.1-4, pp. 33-40, ISSN 1872-8081

Zeindl-Eberhart, E., Jungblut, P.R., Otto, A., Kerler, R., Rabes, H.M. (1997). Further characterization of a rat hepatomaderived aldose-reductase-like protein-organ distribution and modulation in vitro. European Journal of Biochemistry, Vol.247, No.3, (August 1997), pp. 792–800, ISSN 0014-2956

Zhi-Hua, C. & Etsuo, N. (2006). 4-Hydroxynonenal (4-HNE) has been widely accepted as an inducer of oxidative stress. Is this the whole truth about it or can 4-HNE also exert protective effects? IUBMB Life, Vol.58, No.5-6, (May-june 2006), pp. 372–373, ISSN 1521-6543



Colorectal Cancer Biology - From Genes to Tumor
Edited by Dr. Rajunor Ettarh

ISBN 978-953-51-0062-1
Hard cover, 446 pages
Publisher InTech
Published online 10, February, 2012
Published in print edition February, 2012

Colorectal cancer is a common disease, affecting millions worldwide and represents a global health problem. Effective therapeutic solutions and control measures for the disease will come from the collective research efforts of clinicians and scientists worldwide. This book presents the current status of the strides being made to understand the fundamental scientific basis of colorectal cancer. It provides contributions from scientists, clinicians and investigators from 20 different countries. The four sections of this volume examine the evidence and data in relation to genes and various polymorphisms, tumor microenvironment and infections associated with colorectal cancer. An increasingly better appreciation of the complex inter-connected basic biology of colorectal cancer will translate into effective measures for management and treatment of the disease. Research scientists and investigators as well as clinicians searching for a good understanding of the disease will find this book useful.

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen