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RESEARCH PAPER

TGFβ1 expression in colonic mucosa: modulation by dietary lipids

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Abstract Transforming growth factor beta1 (TGFβ1) is fundamental to maintain the intestinal epithelial cell homeostasis through its control action on cell proliferation, differentiation and apoptosis. TGFβ1 dysregulation has been observed in several chronic human diseases, including ulcerative colitis, Crohn's disease and colon carcinoma. In the first two conditions, a marked oxidative stress is consistently present, while in the third one, levels of reactive oxygen species tend to be significantly lower than in the surrounding normal tissue. Lipid-derived compounds such as the aldehyde 4-hydroxynonenal (HNE) or cholesterol oxidation products (oxysterols) were shown able to induce expression and synthesis of TGFβ1, an event which can be detrimental or beneficial, essentially depending on its actual intensity. Understanding how specific dietary lipids may influence the complex molecular signaling underlying this cytokine expression, may provide new indications for therapeutic and preventive strategies in inflammatory bowel diseases and colon carcinoma.

Keywords TGFβ1 · Cell signaling · Inflammatory bowel diseases · Colon cancer · 4-Hydroxynonenal · Polyunsaturated fatty acids

Introduction

The colon mucosa provides a physiological barrier against potentially pathogenic components of the diet and intestinal flora present in the lumen. The colon epithelial cells counteract these insults through a highly complex network of cell signals that control intestinal immune response, inflammatory status, and mucosal wound healing. Persistent inflammation, due to bacterial pathogens or genetic predisposition, induces massive recruitment of inflammatory cells, which lead to derangement of mucosal barrier. Cytokines, chemokines and antimicrobial mediators, such as prostaglandins and eicosainoids derived from lipid metabolism, are actively involved in this process.

Dietary lipids have an important pathophysiological impact on mucosa function: they influence membrane fluidity, which may alter cell signaling from the receptor system, immune response, inflammatory reactions and cell death [70, 78]. A large body of evidence from epidemiological studies indicates that dietary lipids, such as polyunsaturated fatty acids (PUFAs) or cholesterol oxidation products, and intestinal microflora, are the main responsible for the production of oxidized species in the colon [78, 115]. Excessive oxidative cell reactions together with reduced antioxidant tissue defenses are prominent features in the pathogenesis of several chronic gastrointestinal diseases [13, 46].

Certain lipid oxidation products may enhance the activity of several inflammatory cytokines, including transforming growth factor β1 (TGFβ1), and thus these products may exert pro-inflammatory and pro-fibrogenic effects within the colon mucosa [54, 86]. TGFβ1 is normally expressed in endothelial, hematopoietic and mesenchymal cells, and it is well known to be a regulator of proliferation, survival, differentiation and apoptosis in different cell types.

TGFβ1 regulates angiogenesis and vascular remodeling by exerting a balanced effect on the stimulation or inhibition of endothelial cell proliferation [9]. This cytokine is

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crucial in regenerating and remodeling damaged tissues and organs during wound repair: it promotes the deposition of extracellular matrix proteins, activates the proliferation of fibroblasts or smooth-muscle-like cells (myofibroblasts) and inhibits the growth of normal epithelial cells [90]. TGFβ1 influences epithelial cell proliferation by blocking the cell cycle at the G1 phase and inducing the activation of cell signals of differentiation and apoptosis [3, 66, 97]. Thus, aberrant TGFβ1 signaling might be expected to crucially favor many pathological conditions.

The important role of lipids as mediators in a wide range of cellular responses, including cell growth, differentiation and apoptosis, and the close connection with the abnormal biological activities of TGFβ1 observed in different intestinal diseases, stress the hypothesis of the involvement of dietary lipids in the modulation of TGFβ1 cell signaling.

TGFβ1 cell signaling

TGFβ1 is secreted in the extracellular space by most mammalian cells in the form of a latent non-active complex, bound to the latency-associated pro-peptide (LAP) and with a latent TGFβ binding protein (LTBP): these latent proteins increase the stability of TGFβ1. The non-active form is activated in two steps: its recognition by the binding to extracellular matrix proteins and a proteolytic cleavage which involves plasmin, calpain or catepsin.

The cell response to activated TGFβ1 mainly involves two transmembrane serine/threonine kinase receptors, three types of receptors I, TβRI (also termed ALKs, activin receptor-like kinases), ALK5/RI, ALK2, ALK1, and the type II, TβRII. TGFβ1 binds TβRII, which recruits TβRI into a heterotetramer; once the heteromeric complex is formed, TβRII phosphorylates a domain of TβRI, which, in turn, phosphorylates specific transduction proteins.

The receptor complex propagates signals through different intracellular mediators, mainly with the Smads. Smads are proteins with specific functional properties, which are activated by the different ligands belonging to the species- and cytotypes-dependant TGFβ family: R-Smads (receptor-activated Smads 1–2–3–5–8), Co-Smad 4 (common-partner Smad4) and I-Smads (inhibitory Smads 6 and 7). TGFβ1 selectively activates both Smad2 and Smad3, which are phosphorylated by receptor kinases. Upon phosphorylation, R-Smads form a heterotrimeric complex with Smad4, which translocates into the nucleus where it can recognize specific DNA sequences (Smad binding elements, SBE) in the promoters of several target genes, acting as transcriptional co-modulator [65, 106]. Cell-specific factors dictate the choice of Smad target genes; indeed, a wide range of proteins cooperate as Smad binding partner (Sp1, E2F4/5, Max, ATF2, ATF3, c-Fos, c-Jun, JunB, JunD etc.) for specific promoters in order to provide high sequence specificity [35, 64].

The inhibitory Smad7 participates in a negative feedback loop to control TGFβ1 responses by interfering with the activation of R-Smads, via proteolytic degradation or dephosphorylation of TßRI [45, 75, 102]. Up-regulation of Smad7 may be induced by the stimulation of other signaling pathways, interferon-γ through Jak1/Stat1 pathway, tumor necrosis factor-α and interleukin-1 through NF-kB/RelA, and EGF via MAPKs [22, 112].

Once in the nucleus, the activated Smad complex can also recruit either transcriptional co-activators (CBP/p300), or co-repressors (TGIF, SKI, SnoN), which can balance opposing effects, depending on the cell type and conditions, implying that cell-specific factors influence the choice of Smad target genes [64].

Although many experimental data demonstrate that Smads are critical for the antiproliferative activity of TGFβ1, other signaling pathways are involved and cooperate either in a Smad-dependent or in a Smad-independent mode, i.e. ERK (extracellular signal related kinase), JNK (c-Jun N-terminal kinase), or p38 MAPKs (mitogen-activated protein kinases) [23]. The presence of an additional Smad4-independent response to TGFβ1 has been validated by the studies performed on a mutant colon adenocarcinoma cell line [36]. ERK, JNK, and p38 MAPK play a key role in the specificity of TGFβ1-mediated different effects. For instance, activation of JNK can enhance Smad signaling through its direct phosphorylation or the activation of c-Jun (a constituent of Activator Protein Binding 1, AP-1) and ATF-2 (activating transcription factor-2) [23, 111]. TGFβ1 signal has also been associated with activity of members of the Rho family GTPases, which are involved in organization of the actin cytoskeleton, myofibroblast transdifferentiation and colon cancer invasion [24, 31]. As proposed for wound healing, chronic inflammation and development of skeletal muscle, these different pathways could interact throughout JNK activation [69, 105]. On the other hand, the MAP kinase kinase kinase TAK1 (TGFβ-activated kinase), which initiates ERK and p38 MAPK pathways, can mediate the activation of redox sensitive transcription factors AP-1 or NFkB (Nuclear Factor kB) [20, 22, 98].

How TGFβ1 signal influences colon mucosa function

In normal intestinal epithelium, the cell cycle output induced by TGFβ1 allows subsequent differentiation of stem cells, which constantly migrate from the bottom of crypts towards the surface of the epithelium, assume the enterocyte non-proliferating differentiated phenotype, undergo apoptosis and shed into the lumen: this

organization allows a continuous layer of cells with functional maturity to be maintained at the surface epithelium. On the basis of these functions, TGFβ1 is expressed as a gradient along the normal colon crypts, with the highest levels on the tip of the villi, whereas the lowest level is associated with the base crypt compartment [31, 92].

A study by Kushiyama and coworkers [52] reported site dependency of TGFβ1 production along the different colon tracts, it being higher in the proximal colon than in the distal portion; actually, TGFβ1 appears more concentrated in the colonic tract where stool are compacted and the mucosa is constantly exposed to detrimental substances with a high risk of inflammation and development of colorectal cancer.

Maintenance of correct gut function requires a complex multifunctional activity of TGFβ1, which involves different components of the entire signaling cascade and induces specific cell responses. TGFβ1 has a central immune-regulatory role: in particular it acts as an inductor of oral tolerance against orally-administered food antigens, by influencing Th1, Th2, and Th3 lymphocyte responses [76]. Studies on TGFβ1 knockout mice have shown that TGFβ1 signaling deficiency within hemopoietic cell is sufficient to maintain T cells activated and to develop severe inflammatory gastrointestinal diseases [39, 41].

TGFβ1 blocks T cell proliferation essentially through cell cycle arrest, either directly or by inhibiting interleukin-2 activity [4, 116]. It also interferes with T cell differentiation, by suppressing transcription factors, which are the master regulatory T cell commitments, such as T-bet or GATA-3 [40]. Recently, it has been shown that specialized populations of $CD4^+/CD25^+$ regulatory T cells (Treg) control intestinal inflammation induced by immune response, via IL-10- and TGFβ1-dependent mechanisms. In addition, naïve peripheral T cells may acquire a Treg phenotype under the influence of TGFβ1 [8, 33, 74].

The continuous intestinal cell replacement is closely related to the cells' interaction with extra-cellular matrix (ECM) and requires rigorous control of ECM synthesis processes: a lack of correct cell anchorage to ECM triggers a specific type of apoptosis, the so-called anoikis. TGFβ1 is involved in regulating cell-matrix and cell–cell adhesion, whereby it may additionally control appropriate epithelial-cell differentiation [113]. Furthermore, TGFβ1 stimulates chemotaxis and proliferation of stromal fibroblasts, which contribute to wound repair and play a role in the maintenance of tissue homeostasis, as well as in intestinal mucosa integrity [90]. Close to the basal surface of epithelial cells, fibroblasts are present that express features of smooth muscle, the so-called myofibroblasts. Myofibroblasts are able to regulate a number of epithelial functions and to maintain mucosal tolerance and $CD4^+$ T cell responses. TGFβ1 has been implicated in myofibroblast differentiation and activation [91, 96].

During angiogenesis, TGFβ1 controls the proliferation, migration and differentiation of smooth muscle and endothelial cells. For instance, the different types of TβRI presumably account for the settled TGFβ1 signal induction, in particular ALK 5/RI is ubiquitously and more abundantly expressed, whereas ALK1 is endothelium specific. In endothelial cells ALK 5/RI inhibits migration and proliferation, unlike ALK1, which induces these two pathways: thus TGFβ1 regulates vascular homeostasis by producing a balance between ALK 5/RI and ALK1 signaling [9, 53]. Other accessory TGFβ1 receptors have been identified, the betaglycan and endoglin type III receptors, which are transmembrane molecules with different roles: betaglycan facilitates the binding of TGFβ1 to TβRII, while endoglin (also named CD105) is a glycoprotein which binds ligand only when it is already associated to TβRII and acts as regulator of TGFβ1/ALK5 signaling on endothelial homeostasis [32, 37, 83].

TGFβ1 controls the growth of normal epithelial, endothelial, smooth muscle and lymphoid cells, essentially by blocking the cell cycle at the G1 phase, by down-regulating the c-Myc oncogene and concomitantly up-regulating p15INK4B, p27KIP1 and p21CIP1 cell cycle inhibitors [8, 100]. It has been shown that c-Myc represses p15INK4B by interacting with a zinc-finger protein Miz-1, which recognizes a p15INK4B core-promoter element; in response to TGFβ1, the activated Smad complex binds directly to the p15INK4B promoter, thus relieving p15INK4B from Myc interaction. Similarly, p21CIP1 expression may be up regulated by TGFβ1-induced activation [34, 66, 80].

Studies on colorectal carcinogenesis indicate that TGFβ1 blocks the survival pathway Akt/PKB (a serine/threonine kinase activated by phosphatidylinositol-3-kinase) and its induction of anchorage-independent growth in gut epithelial cells, by suppressing NFkB activity, by stabilizing its inhibitor factor IkB, and inducing the cells to anoikis [16].

TGFβ1-induced apoptosis is mainly mediated by cross talk between Smad and JNK [11]. Activated Smads enhance p53 signaling by forming a direct p53-Smad complex that activates a specific Smad-binding DNA sequences [29, 35]. Furthermore, TGFβ1 appears to positively interact with apoptotis induced by the Fas ligand, which involves the sequence of adapter molecules Daxx and ASK1 (apoptosis signal-regulating kinase1) and activates apoptotic signals through JNK [82]. Conversely, Akt/PKB protects against TGFβ1/JNK-dependent apoptosis through the phosphorylation and sequestration of ASK1 [97].

Dietary lipids

In the twentieth century, due to lifestyle's modifications, our Western diet has undergone marked changes, the major difference being an increased fat intake. In particular, the amount and the type of dietary lipids, especially polyunsaturated fatty acids (PUFAs) and cholesterol/cholesterol esters, have been increased. These dietary changes resulted to be associated to the increase of chronic inflammatory, autoimmune and vascular diseases, as well as cancer development [68, 70, 93, 94, 99].

Lipids play structural and regulatory functions which may influence cell membrane fluidity, receptor mobility and functions, and relevant signal transduction. They may act as mediators of cell responses including growth, differentiation and apoptosis [12, 27, 70, 71].

PUFAs are found in animal and vegetable oils, fish and meat, and consist of the essential fatty acids omega-6 (linoleic acid, LA) and omega-3 (linolenic acid, ALA), which are the precursors of membrane omega-6 arachidonic acid (AA), omega-3 eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids. Mammals are unable to synthesize LA and ALA, which, therefore, must be provided in the diet. Once absorbed, LA and ALA are enzymatically converted by microsomal elongases and desaturases into AA and EPA. EPA can then be transformed into DHA.

AA and EPA are immediately esterified to membrane phospholipids, from which they can be mobilized by phospholipase A2 (PLA2) and oxidized by cyclooxygenases (COXs) or lipoxygenases, to form different types of eicosanoids, i.e. prostaglandins (PG) and leukotrienes (LTB). Generally, eicosanoids generated from AA have different functions than those generated from EPA. For instance, PGE2 and LTB4 omega-6 products have pro-inflammatory action as inducers of platelet aggregation, leukocyte chemotaxis and adherence, while PGE3 and LTB5 omega-3 products have opposing anti-inflammatory effects.

Since both omega-3 and omega-6 fatty acid metabolism requires the same enzymes, an excess of one fatty acid family may interfere competitively with the metabolism of the other, e.g. significantly reducing its incorporation into tissue lipids. Thus, a prevailing intake of omega-6 rather than of omega-3 PUFA with the diet can influence membrane phospholipid composition, eicosanoid synthesis and related inflammatory, immune and hormonal responses. The intake of certain PUFAs induces a variations in the fatty acid composition of immune cells and appears to be related to the variability of immune-cell functions [47, 108]. For example, markedly increased consumption of ALA or fish oil decreases T lymphocyte proliferation [107]. A DHA-rich diet could ameliorate Th1-mediated inflammation and reduce colon tumorigenesis, because this omega-3 fatty acid alters the balance between CD4+ Th1 and Th2 by directly suppressing Th1 cell development. Th1 cells produce specific inflammatory cytokines, such as IL-2 and interferon-gamma, which are involved in cell-mediated immunity and are predominant in Crohn's disease [18]. This feature underlines the importance not only of the amount of fat, but also of the ratio between omega-6 and omega-3 PUFAs ingested.

North American countries and Northern and Western Europe consume diets containing large amounts of red and processed meat compared to Eastern European and in Mediterranean countries, where diets contain more fruit, vegetables, fish and olive oil. Several epidemiological and pathological studies have provided evidence that "Westernization" of dietary and nutritional practices is an important determinant in inflammatory bowel diseases, colorectal polyps and colorectal cancer. Numerous studies have underlined the importance of the specific fatty-acid composition in the pathogenesis of these chronic diseases [17, 28, 70, 93, 104].

It has been clearly documented that LA and AA, among cell-membrane phospholipids, are substrates for the synthesis of a range of biologically-active mediators, including prostaglandins, thromboxanes, and leukotriene, which convey inflammatory responses by modulating IL-6, IL-1β, TNF-α, TGFβ1 and interferon-gamma expression [2, 15, 42, 108]. Furthermore, lipids represent one of the most important cell targets of oxidative stress. In fact, AA is a selective substrate for oxidative breakdown of lipid cell membranes, defined as lipid peroxidation, which leads to the formation of a variety of molecules also able to exert toxic and mutagenic effects [73, 79]. Lipid peroxidation can be initiated by different free radicals and is characterized by a rise of both reactive oxygen species (ROS) and radical intermediates, which often reduce cellular antioxidant defenses and cause an imbalance between oxidative and reductive reactions. This process is commonly known as oxidative stress, and may be due to different causes: unbalanced diet, with reduced antioxidant intake or increased omega-6 PUFA intake, intestinal infections, which recruit activated phagocytes, environmental and genetic factors, including chronic inflammation of the gut.

Enhanced oxidative degradation of membrane lipids involves cleavage of lipid hydroperoxides and leads to the formation of reactive aldehydes, including malonaldehyde (MDA) and 4-hydroxynonenal (HNE). HNE, due to the presence in its molecule of the hydroxyl group near the double bond, readily reacts with thiol- and amino-groups, by this way influencing the function of biomolecules, for example causing DNA damage (with oxidation of guanine to form 8-oxo-7,8-dihydro-2′-deoxyguanosine), changes in the structure of membrane phospholipids and proteins, and enzymatic inactivation.

In the last few years, oxygen-derived oxidants have gained attention as potential signaling molecules under not toxic conditions. In fact, at concentrations compatible with the physiology of the cell, ROS and related lipid oxidation products may influence cell differentiation and proliferation by activating transduction signal molecules and redox-sensitive transcription factors, such as AP-1 and NFkB, involved in the modulation of cell viability and function [62, 89].

The hydroxyalkenal HNE, which derives from oxidative degradation of AA, possesses numerous biochemical effects, some with demonstrated biological impact. This aldehyde is a relatively stable molecule, which can diffuse to sites distant from its origin, by this way propagating the initially local injury. An increasing bulk of experimental evidence points to HNE as a candidate molecule for a role in the pathogenesis of numerous inflammatory and degenerative processes, including atherosclerosis, Alzheimer's and Parkinson's diseases, liver fibrosis, glomerulosclerosis, gastrointestinal diseases and cancer [26, 88]. In addition, various studies have focused on the biological impact of lower concentrations of this aldehyde, which is produced in physiological conditions as a product of the normal renewing process of PUFAs in cell plasma membranes. Of note, in concentrations ranging from 0.1 to 10 μM, HNE does not appear to be merely a toxic product of lipid peroxidation; on the contrary, it also exerts several important biochemical effects, including modulation of different signaling pathways [89]. HNE can control growth and differentiation, cell cycle progression and apoptosis. Indeed, this molecule may be considered as antiproliferative, being able to regulate genes encoding for other molecules such as c-myc, c-myb, cyclins, p21 and TGF-β1 [7, 50, 54, 88, 110].

Besides PUFA oxidation products, also oxysterols, i.e. 27 carbons cholesterol oxidation products, appear able to trigger and sustain cell signaling. These compounds may be absorbed with the diet or originate endogenously. Exogenous oxysterols derive from cholesterol degradation and oxidation after prolonged storage or cooking of foods rich in cholesterol (cheese, eggs, meats, powdered milk). Endogenous production of oxysterols may partly occur within tissues, through non-enzymatic oxidation of cholesterol involving oxygen species, or via enzymatic catalysis. These oxidized compounds may exert a number of biochemical actions. They are thus now considered to be definitely implicated in the pathogenesis of several chronic human diseases, especially those involving the alteration of cholesterol homeostasis (for a review see [1, 57, 99, 117]).

Dietary lipids and their oxidative modifications undoubtedly mediate specific cellular and molecular responses and gene expression, processes that are crucial in the promotion of chronic inflammation, fibrosis and programmed cell death.

Relation between TGFβ1 signaling and oxidized lipids in human pathology

The intestinal epithelium is continuously renewed by a dynamic process, which covers the phases of cell proliferation from the bottom of the crypts, migration, differentiation and shedding into the lumen by anoikis. Maintenance of proper cell functioning requires strong defenses against environmental insult. However, continuous exposure of the intestinal mucosa to inflammatory agents, such as bacterial cell products, inflammatory cytokines, or genetic disorders, may derange proper intestinal cell function. Increased free-radical production, generally resulting from reactive oxygen and nitrogen intermediates generated by infiltrating phagocytic cells, high dietary lipid intake, or reduced antioxidant defenses, could maintain an active inflammatory status, which sustains intestinal damage and predisposes to colon cancer [43, 49, 70, 109]. Furthermore, lipid oxidation products themselves induce the expression and synthesis of inflammatory and fibrogenic cytokines, and TGFβ1 has been proved to be one of their main targets [86, 87].

Inflammatory bowel diseases

Oxidative stress and its lipid oxidation products may influence host susceptibility to inflammatory bowel diseases (IBD), such as ulcerative colitis and Crohn's disease. IBD is a remitting relapsing disorder of the gastrointestinal tract, characterized by chronic inflammation in parts of the gastrointestinal tract. The etiology of these diseases is still unknown**,** but dysfunction of the immune system, as well as genetic and environmental factors, may well play a role. The inflamed intestinal mucosa is characterized by uncontrolled release of cytokines and of oxidative species, which chiefly arise from respiratory burst of activated phagocytes. The dysfunction of TGFβ1 signaling is widely involved in the overproduction of inflammatory mediators in IBD [44, 60, 72]. In the advanced stages of IBD, fibrosis is the major complication; it manifests as strictures in Crohn's disease and as colonic shortening in ulcerative colitis: fibrosis is mediated by intestinal fibroblasts, located at the interface between the epithelium and the lamina propria, which achieve the myofibroblast phenotype in the presence of TGFβ1.

Many studies have associated TGFβ1 production and lipid oxidation products with the initiation and progression of fibrosis in different organs: the major effect of oxidative stress on the gene expression of this cytokine appears to be an important mechanism whereby it promotes connective tissue deposition.

Experiments "in vitro" performed by Leonarduzzi and colleagues [56] have shown that the incubation of murine and human macrophages with different oxysterols, matching a concentration consistently detectable in the plasma of hypercholesterolemic subjects, induced up-regulation of the expression and synthesis of TGFβ1 by these cells. Different types of oxysterols may influence cell signaling in various ways. 25-Hydroxycholesterol or 7-beta-hydroxycholesterol have been found cytotoxic for Caco-2 human colon carcinoma cell line [78], whereas lower concentrations of 25-hydroxycholesterol associated with IL-1beta induced production of inflammatory IL-8 in the same cells [6]. On the other hand, a mixture of oxysterols, obtained by cholesterol heating, that mimics the concentration of these compounds in cooked foods, increased the activation of liver X receptors (LXRs), which are important regulators of cholesterol catabolism and lipid metabolism, thus negatively influencing inflammatory cell response [85, 120]. A similar mixture of oxysterols can induce apoptosis in Caco-2 cells by increasing caspase-3 activity (Biasi and colleagues, unpublished data).

Different studies from our laboratory have proved a direct correlation between AA degradation products, such as HNE and MDA, and TGFβ1 overproduction in fibrotic diseases, i.e. liver fibrosis, atherosclerosis and Crohn's disease. Notably, in plasma from Crohn's patients, very large amounts of proteins modified by biochemical interaction with HNE and/or with MDA were detected. Such HNE/MDA-protein adducts appear to be reliable markers of oxidative reactions [10, 19, 86, 87, 118]. Pathophysiological amounts of HNE induce TGFβ1 expression and synthesis in promonocytic and macrophagic cells [54] and augment both expression and synthesis of procollagen type I in primary cultures of human stellate cells [81]. The signaling pathways adopted by this aldehyde in modulating these genes, and its related hydroxyalkenal compounds, most likely imply the activation of kinases involved in the regulation of the redox-sensitive transcription factor AP-1 [55]. More recently, our studies on the cross-talk between HNE and TGFβ1 signals have identified a direct influence of HNE on the activation of Smad proteins, which are considered the main transduction proteins related to TGFβ1-mediated cell response [11].

The importance of aldehydic products of PUFA degradation in colon diseases was remarked by epidemiologic studies on fatty-acid profiles in IBD patients, with the evidence of a net decrease of n-3 fatty acids in Crohn's disease [51, 59, 103]. Increasing dietary intake of n-3 PUFA has been employed to reduce high levels of inflammatory AA metabolites and lipid peroxidation products. Notwithstanding various attempts, at present there are not sufficient data to demonstrate with certainty that treatment with n-3 PUFA supplementation could induce total remission of the disease (for a review see [59]).

Colon cancer

It is well established that many tumors arise from sites of infection and chronic irritation as well as inflammation. The most widely studied and best established of these links are colon carcinomas associated with IBD. In fact patients with IBD are at increasing risk of colorectal cancer development, which may be summarized by three main steps of molecular and clinical features: inflammation, dysplasia and carcinoma [21, 43, 101].

During chronically inflamed intestine, persistent oxidative reactions, with an overproduction of lipid peroxidation end-products such as aldehydes and epoxides, may be tumorigenic by virtue of their ability to produce chemical adducts to DNA, oxidation of DNA bases or errors in DNA repair. These DNA changes lead to mutations in specific genes, some of which are critical in the regulation of colon cell proliferation, survival and migration [38, 63, 84].

Because of the crucial role of TGFβ1 in the negative regulation of cell proliferation, somatic mutations and loss of the expression of genes that are involved in its signaling can induce errors in cell growth regulation, by this way favoring tumor formation [5, 14, 30, 61, 64].

A large percentage of pancreatic cancers and colorectal cancers have mutations in some component of this pathway. Alterations in type I and II TGFβ1 receptors are frequently observed in human colon adenocarcinoma [77, 114]. However, no consistent relationship between distribution of the receptors, degree of tumor dedifferentiation and TGFβ1 tissue concentration may exist [10, 119]. Smad4 mutations have been identified in a significant portion of pancreatic-, common bile duct-, and colorectal-cancers [48, 67], while Smad2 gene alterations are limited to a small number of colorectal cancer [58, 95].

During early stages of carcinogenesis, oxidative reactions can cause changes in proliferation regulatory pathways which involve TGFβ1. On the contrary, once transformed, cancer cells become generally resistant to oxidative stress and their susceptibility to lipid peroxidation decreases. Major reasons for this behavior are an increased cholesterol-dependent rigidity and a decreased availability of the peroxidative substrates arachidonic and linoleic acids in tumor membranes [25].

A direct correlation between diminished extent of membrane lipid peroxidation and TGFβ1 tissue content has been observed "in vivo" in human colon adenocarcinoma, in which a strong association between low membrane lipid peroxidation and decreased TGFβ1 expression exists, inversely related to the tumor malignancy. Indeed, in the

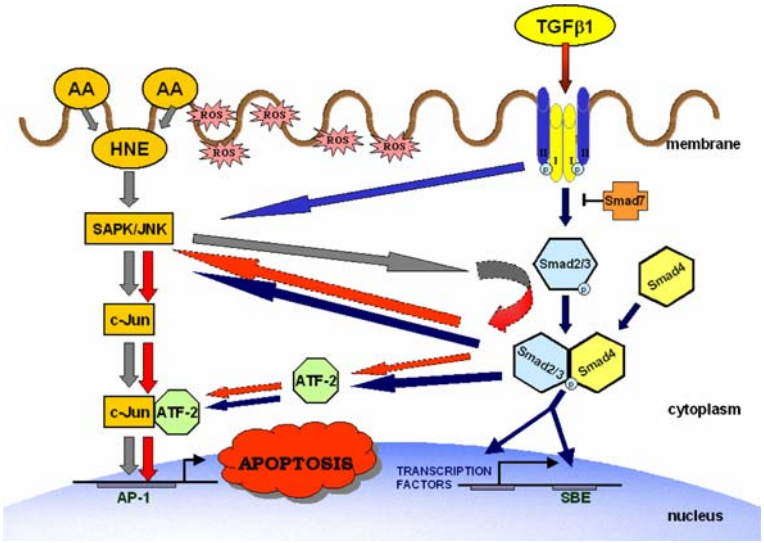

Fig. 1 Integration of the TGFβ1 apoptotic signal by the lipid oxidation end-product 4-hydroxynonenal. TGFβ1 induces apoptosis in epithelial cells through up-regulation of redox-sensitive transcription factors, such as AP-1. This signal may be mediated by either a Smad-dependent or a Smad-independent pathway, which in turn involves activation of JNK signaling. A mechanism is suggested whereby arachidonic acid oxidation products, such as HNE, may contribute to enhancing TGFβ1-induced apoptotis. Besides JNK-mediated transduction, HNE acts on Smad proteins, thereby strengthening this pathway. HNE interaction with the Smad pathway can enhance and sustain JNK signaling through a positive loop. This convergence of signals results in an amplification of TGFβ1-induced apoptotis. *AA* arachidonic acid; *HNE* 4-hydroxynonenal; *ROS* reactive oxygen species; *SAPK/JNK* stress-activated protein kinase/c-Jun N-terminal kinase; *ATF-2* activating transcription factor-2; *TGF-β1* transforming growth factor-beta1; *SBE* STAT (Signal Transduction and Activator of Transcription) binding site

plasma of these cancer patients a very low content HNE and MDA-protein adducts was detected [10, 19].

Because of the demonstrated marked ability of HNE to up-regulate expression and synthesis of TGFβ1 [54], and its role as a negative growth controller, the combined low content of HNE and TGFβ1 found in colon cancer tissue, could provide a favorable condition for tumor survival. In fact, transformed cells could be safeguarded from the anti-proliferative control exerted by these molecules. Our recent data on cultivated human colon cells, showed a significant cross-talk between the different signal pathways activated by HNE and TGFβ1, which cooperated to increase apoptotic process [11] (see Fig. 1). In this relation, lipid oxidation products, such as HNE, might be considered among the physiological mechanisms, which can counteract tumor growth: these molecules could efficiently restore and/or amplify the TGFβ1-dependent differentiating and apoptotic pathways that are reduced in cancer cells. Experimental enrichment of colon carcinoma cell membranes with arachidonic acid, the main source of HNE, with the aim to corroborate anti-proliferative pathways is now in progress.

Perspectives and conclusions

It becomes clear that changes in cell membrane lipids may be of primary interest in human pathophysiology. Interestingly, it has been recognized that lipid oxidation derivatives can serve as mediators of specific cellular and molecular responses and gene expression. In this relation, lipid-derived bioactive molecules, such as oxysterols and aldehydes, are strongly implicated in the regulation of TGFβ1 production and function.

Therefore, a suitable modulation of lipid diet with balanced n-6/n-3 PUFA and correct cholesterol intakes, can significantly help in the clinical management of chronic intestine diseases, like inflammatory bowel diseases or colon cancer by making intestinal cells more sensitive to defined cell cycle down-regulators and proapoptotic molecules, such as TGFβ1.

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