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Mitocondrial dysfunction in human colorectal cancer progression

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1. ABSTRACT

The classic association between cancer and mitochondrial dysfunction is actually considered as a role of mitochondria in cellular signalling. It is understood that mitochondria, mitochondrial oxidative damage and NO and H_2O_2 diffusion are involved in the progression of human colorectal cancer. Mitochondria from human colorectal tumors and adjacent non-tumor colon tissues showed a markedly increased oxidative damage with increased contents of TBARS and protein carbonyls. Mitochondrial protein carbonyls was the most sensitive indicator. Oxidative stress and damage was also observed in adjacent non-tumor cells. Mitochondrial activities, as NADH-cytochrome c reductase and cytochrome oxidase, were observed decreased in tumor and in adjacent non-tumor tissue. Cu,Zn-SOD activity decreased by 42% in tumor tissue in the advanced stage as compared with the initial stage, whereas Mn-SOD activity did not change in tumor progression. An increased mtNOS activity (46%) was observed in tumor and non-tumor tissues in the advanced stage of cancer progression. A direct linear relationship between mtNOS and oxidative damage in tumor and non-tumor tissues supports the concept that mitochondrial NO and H_2O_2 diffuse from tumor to adjacent non tumor tissue signaling for cell death as the classic toxohormones.

2. INTRODUCTION

Human colorectal cancer is one of the most frequent cancers in the western countries with important implications in public health and with a frequency that accounts for about 15% of all types of cancer. From an etiologic point of view, different factors have been implicated as cause or risk factor of colorectal cancer; most of them related to dietary habits such an excessive consumption of red meat and fats and a deficient consumption of fruits and vegetables. Other factors recognized as risk factors include modifications of intestinal flora, inflammatory diseases and familial adenomatous polyposis (1-4).

The molecular and cellular changes occuring during the development of the multistage process of carcinogenesis are mediated by a variety of intracellular, extracellular and environmental factors. Prevention of the situations that are recognized as risk factors and prevention of disease development when human colorectal cancer is an early stage are the choice strategies bound to decrease the morbility and mortality of human colorectal cancer. For such purpose, the intracellular scenario of both cancer cells and the cells of the adjacent non-tumor tissue becomes important as the basis for the processes of proliferation and apoptosis involved in cancer progression (5,6).

Mitochondrial dysfunction, increased production of mitochondrial oxyradicals and mitochondrial and cellular oxidative stress and damage have been claimed as playing a central role in the progression of the human colorectal cancer (7,8). In this study we approach such hypothesis by the determination of mitochondrial enzyme activities and oxidation products in tissue samples of human colorectal cancer and of adjacent non-cancer mucosa, at two stages, early and advanced, of colorectal cancer progression.

3. COLORECTAL CANCER AND MITOCHONDRIAL METABOLISM

Otto Warburg pioneered in the 1930 decade the concept of a relationship between cancer and mitochondrial dysfunction. He showed in classic contributions that cancer cells *in vitro* show as a common feature an elevated rate of glycolisis and a decreased rate of aerobic oxidations (9-11). The shift to the glycolitic phenotype of many cancer cells has been exensively recognized at the biochemical and proteomical levels (12); however, at this moment there is no clear evidence about the nature of the impairment of mitochondrial function in cancer cells.

3.1. Bioenergetics and tumor progression

Mitochondria are the ATP-provider powerhouse of the cell. The mitochondrial process of energy trasduction implies oxidation reactions and a phosphorylation reaction and for that reason is called “oxidative phosphorylation”. The oxidation of Krebs cycle derived metabolites $NADH_2$ and succinate is coupled to the vectorial release of H^+ in the P side of the inner mitochondrial membrane with the generation of an electrochemical H^+ gradient or proton-motive force. The H^+ gradient determines the movement of H^+ from the P side to the N side through H^+-ATP synthase that phosphorylates ADP and completes the energy trasduction process. Tumor progression requires an active mitochondrial involvement both in tumor cells and in the adjacent tissue. In the tumor, an increased glycolitic ATP turnover is associated with decreased mitochondrial function and cell proliferation, whereas in the adjacent tissue the mitochondrial dysfunction is associated with apoptosis.

A selective repression of the expression of the beta-catalytic subunit of the H^+-ATP synthase in mitochondria from human colon carcinoma has been reported, concurrent with an increase in the expression of the glycolytic glyceraldehyde-3-phosphate dehydrogenase. The changed proteome would determine the change in cell metabolic profile in cancer, as a molecular renaissance of Warburg hypothesis, suggesting metabolic mechanisms for the resistance to cell death pathways or decreased apoptotic potential of tumor cells (13).

Energy balance is also gaining momentum as an etiologic factor in colon cancer (14,15). An increased risk of colon cancer has been observed associated with increasing body mass index primarily among men (16-19), and physical activity has been shown consistently to reduce the risk of colon cancer (20). One possible mechanism whereby energy balance may be associated with colorectal cancer was suggested as based on the association with insulin and carbohydrate metabolism; however, the risk appears to be minimally influenced by insulin-related genes (21).

3.2. Metabolic pathways in cancer cells

The final catabolic cellular processes occur in mitochondria, as indicated by the location of the enzymes of the Krebs cycle, the beta-oxidation of fatty acids and the urea cycle. The central glycolytic and oxidative pathways and the ATP-producing mechanisms differ in sane and malignant cells by their regulation and dynamics. Fast-growing and poorly-differentiated cancer cells characteristically show high aerobic glycolysis. Other metabolic pathways have been recognized as deficient or different in cancer cells. Mitochondrial aldehyde catabolism, at the origin of a possible acetaldehyde cytoxicity, is circumvented by the synthesis of acetoin, an unusual and non-toxic product, through tumoral pyruvate dehydrogenase. As most of the glycolytic pyruvate is deviated to lactate production, little pyruvate enter a truncated Krebs cycle where citrate is preferentially extruded to the cytosol where it feeds sterol synthesis (12). Cholesterol biosynthesis, occurring by normal pathways in tumors, is deficient in feed-back regulation and in sterol-transport mechanisms.

The changes in cancer cell metabolism allow the cells to survive, proliferate and invade (22) even in hypoxic conditions (23) and the adaptation to low oxygen tensions becomes a crucial step in tumor progression. The generation of ATP from converting glucose to lactate even in the presence of abundant oxygen (24), the so-called Warburg effect, is a characteristic of cancer cell metabolism and an important feature for tumor progression (25). Interestingly, it has been recently reported that activation of mitochondrial lactate uptake by flavone induces apoptosis in human colon cancer (26).

There are a few characteristics of tumor mitochondrial function that are worth to mention. Glutamine is one of the major oxidizable substrates by tumor cells; inside the mitochondrion, glutamine is deaminated to glutamate through a phosphate-dependent glutaminase. Glutamate is then transaminated to alpha-ketoglutarate that enters the Krebs cycle. Glutamine may be completely oxidized through an abnormal Krebs cycle only if a pathway forming acetyl CoA is effective. Cytosolic malate enters mitochondria by specific shuttles and is preferentially oxidized to pyruvate and CO_2 by intramitochondrial NADP-malic enzyme. These and other irregularities of mitochondrial oxidations in tumor cells appear to reflect a complex set of non-random phenotypic changes that are initiated by the expression of oncogenes (12).

Continuously acting glycolysis and gluconeogenesis, fatty acid oxidation and *de novo* lipogenesis constitute futile cycles that maintain the organism in a negative energy balance that may lead to cancer-associated cachexia. Mitochondria-to-nucleus

signaling in tumor cells seems to activate some genes implicated in tumor progression and in tumor cell metastasis. Retrograde regulation between mitochondria and nucleus also renders the cell more resistant to apoptosis (27).

4. FREE RADICAL GENERATION IN HUMAN COLORECTAL CANCER

It has been frequently suggested that the risk of human colorectal cancer is increased by the mutagenic action of free radicals. Colon carcinogenesis appears as a multistep process where oxygen radicals are able to enhance carcinogenesis at all stages: initiation, promotion, and progression (1,2). Superoxide radical (O_2^-) was reported to be a potent promoter of mutagenic activity in the colonic mucosa (28) and oxidative stress and damage are presumed to contribute to the increased cellular mutation rate that accompanies cancer progression (29). Neoplasic cells may overproduce oxygen and nitrogen reactive species and some of these species, likely the uncharged and diffusible ones, may reach adjacent cells facilitating tumor growth and invasion.

4.1. Intracolonic production of free radicals

Intracolonic production of oxygen radicals may be a effective cause in carcinogenesis (2,3). The relatively high concentrations of iron in feces, together with the ability of bile pigments to act as iron chelators, may very well support an efficient HO^- generation by a Fenton chemistry in colon cells. The partially reduced forms of oxygen, O_2^- and H_2O_2, may be equally provided by the same colon cells or by bacterial metabolism. Such free radical generation in feces provides a link in our understanding of the etiology of colon cancer: the oxidation of procarcinogens to form active carcinogens or mitogenic tumor promoters cold be mediated either by fecal HO^- or by secondary peroxyl radicals (ROO^-). Intracolonic free radical formation is consistent with the high incidence of cancer in the colon and rectum, compared to other regions of the gastrointestinal tract, as well as with the correlations of a higher incidence of colon cancer with the amount of dietary red meat, which increases stool iron, and with excessive dietary fat, which increases the fecal content of procarcinogens and bile pigments (30).

4.2. Mitochondrial production of free radicals

The generation of reactive oxygen species by the mitochondrial electron transfer chain has been related to of multiple harmful molecular processes, including lipid and protein oxidation, disruption of signaling pathways and transcription factor modulation, and oxidative cellular damage. In physiological conditions and in both cancer cells and in non-cancer cells, there is a continuous mitochondrial production of reactive oxygen and nitrogen species (O_2^-, H_2O_2, NO, $ONOO^-$, HO^-, ROO^-, and $^1O_2$) (31-33), which are able to react and damage biomolecules leading to cumulative oxidative damage (34). Superoxide anion radical is generated as a byproduct of the respiratory chain electron transfer, mainly by the autoxidation of ubisemiquinone (32,35). Mitochondrial nitric oxide (NO) is the product of the enzymatic action of mitochondrial nitric oxide synthase (mtNOS) (34,36), a specialized nitric oxide synthase NOS) (37) that carries out a classical NOS reaction, requiring NADPH, arginine, O_2 and Ca^{2+}/calmodulin for enzyme activity (38). Mitochondria, the main cellular site of O_2 uptake and oxyradical generation, are also the main target of oxyradical-mediated damage.

The increased steady state concentrations of any of the reactive oxygen or nitrogen species constitutes the chemical basis of the biological situation of oxidative stress (39), and the persistence of oxidative stress and damage is understood to contribute to cancer progression. Human colorectal carcinoma, as a chronic inflammatory process, overproduces nitrogen and oxygen reactive species that damage normal adjacent mucosa that thereby facilitate tumor growth and invasion (40).

5. MITOCHONDRIAL OXIDATIVE DAMAGE IN HUMAN COLORECTAL CANCER

The free radical chain reactions started by O_2^- and NO and involving a series of reactive oxygen and nitrogen species is capable of damaging mitochondrial membranes, proteins and DNA. Thiobarbituric acid reactive substances (TBARS), protein carbonyls, and 8-hydroxy-deoxyguanosine (8-HO-dG), are the usual markers of oxidative stress as by-products of free radical-mediated oxidation of cell components.

5.1. Mitochondrial lipid and protein oxidation in patients with colorectal cancer

We examined samples from 60 patients operated of colorectal cancer at the Hospital Universitario Puerta del Mar and the Military Hospital of Cadiz (the study was approved by the Ethics Committee on Human Experimentation in both Hospitals, and the patient informed consent was given in agreement with the Helsinki Declaration).

Mitochondria from human colorectal cells were in an oxidative stress situation as compared with the cells from the adjacent tissue (Table 1). The increased oxidative damage, measured as TBARS and protein carbonyl contents, was markedly increased (2.2-2.4 times) in mitochondria from tumor tissue in the advanced stage, as compared with the almost normal situation of the cells adjacent to tumors in the initial stage. Interestingly, an increased mitochondrial oxidative, in the range of 1.7-2.5 times, was observed in the non-tumor tissue adjacent to the tumors in the advanced stage of evolution.

Patients with colorectal cancer also showed a systemic oxidative stress during cancer development. The total level of antioxidant in plasma decreased while lipid peroxidation products increased. Patients showed a significant increase in plasma malonaldehyde and 4-hydroxynonenal levels (41-45). Lipid peroxidation products and arachidonic acid peroxy-metabolites have been

Table 1. Oxidative damage in mitochondria of tumor and non-tumor adjacent tissue from patient in initial and advanced stages of human colorectal carcinoma

	Initial stage		Advanced stage	
	Non-tumor	Tumor	Non-tumor	Tumor
TBARS	3.2 ± 0.3	3.6 ± 0.3	5.3 ± 0.5	6.9 ± 0.5[1]
Protein carbonyls	38 ± 4	47 ± 4	94 ± 8[1]	91 ± 8[1]

The initial stage group is referred to patient with pathological diagnosis of human colorectal adenocarcinoma in A-B Dukes stages and I-III TNM classification (n = 33). The advanced stage group corresponded to C-D Dukes stages and III-IV TNM classification (n = 27). An amount of 150-250 mg of tumour and adjacent histologically normal mucosa (at least 5 cm from the tumour) was used for isolation of mitochondria, that was performed as described (78). The mitochondrial content of thiobarbituric acid-reactive substances (TBARS) and of protein carbonyls were determined as described by Fraga *et al.* (79); Oliver *et al.* (80) and Navarro *et al.* (58). Values are means ± S.E.M. in pmol /mg mitochondrial protein. Differences between groups were analyzed by the Student-Newman-Keuls post hoc test after significant one way ANOVA. [1]$p < 0.05$ for advanced stage ≠ initial stage.

implicated in genotoxicity, tumor initiation, and promotion (46).

Nakahara and Fukuoka in 1958 reported that a chemical fraction concentrated from human colorectal cancers depressed liver catalase activity within a day after injection in mice (47). The original observation was followed by a series of reports on a heterogeneus group of small peptides isolated from a number of tumors that were generically named toxohormone and that shortly after injection into mice caused cachexia (48), similarly to the situation of patients with cancer. Toxohormones increase oxidative damage with increased lipid and protein oxidation in tissue different of tumoral tissues, as adjacent non-tumor tissue or distant tissues. Boveris *et al.* (49) observed that tumor-bearing mice exhibit increased *in situ* liver chemiluminescence indicating that toxic metabolites are released by tumors to lymph and plasma.

Therapeutic approaches using antioxidants seem to match a decrease in oxidative stress and damage with an improvement in cancer progression. In this sense, it was proposed that beta-carotene antagonizes the effects of eicosapentaenoic acid in colon cancer cell growth and lipid peroxidation (50). It has been also suggested that the redox regulation of NF-kappaB induced by beta-carotene is involved in the growth-inhibitory and pro-apoptotic effects of the carotenoid in tumor cells (51). Moreover, there are interesting reports that suggest vitamin E and analogues as effective antineoplastic agents (52-54).

5.2. mtDNA mutations and colorectal cancer

Alterations in the expression of the mitochondrial DNA (mtDNA) encoded polypeptides required for electron transfer and ATP synthesis are a characteristic of cancer cells. Mitochondrial DNA has been strongly related to carcinogenesis because of its high susceptibility to mutations and limited repair mechanisms in comparison to nuclear DNA. Since mtDNA lacks introns, it was considered that most mutations will occur in coding sequences and the subsequent accumulation of mutations may lead to tumor initiation. The mitochondrial genome is dependent upon the nuclear genome for transcription, translation, replication and repair, but the mechanisms for how the two genomes interact and integrate with each other are poorly understood (55). Mitochondrial DNA encodes subunits of respiratory chain enzymes: seven of complex I, one of complex III, three of complex IV and two of complex V. Alterations in the expression of mitochondrial encoded respiratory chain subunits have been detected in primary tumor samples from colorectal cancer patients as well as in patients with pre-malignant familial polyposis coli syndrome. Analysis of the mitochondrial genome in colorectal cancer cell lines also revealed abnormalities (56,57).

6. DECREASED RESPIRATORY CHAIN ACTIVITIES IN HUMAN COLORECTAL CANCER

A few enzymatic activities, that encompass the whole function of the mitochondrial electron transfer chain, were determined in this study in human colorectal cancer mitochondria. The referred activities were: NADH-cytochrome c reductase (NADH => cyt c) that measures the activities of complexes I and III, succinate-cytochrome c reductase (succinate => cyt c) that assays the activities of complexes II and III, and cytochrome oxidase (cyt c => O_2) that gives complex IV activity. The activity of NADH-cytochrome c reductase was markedly lower (45-31%) in tumors and also in the adjacent non-tumor tissue in the advanced stage (Table 2). Considering that the same samples did not show any significant change in succinate - cytochrome c reductase activity, the result is considered an indication of a specific change in Complex I (NADH–dehydrogenase) activity (58). Tumor cytochrome oxidase activity was also markedly decreased (61-38 %) in tumors and in adjacent non-tumor tissue in the advanced stage.

The specific damage to Complex I in neoplastic cells was early observed by Granger and Lehninger (59) and mitochondria from rat colon tumors induced by of 1,2-dimethyl-hydrazine exhibited reduced NADH-cytochrome c reductase activity (45). Moreover, Sun *et al.* (60) reported that cytochrome oxidase and succinate dehydrogenase activities were lower in human colon carcinoma, as compared with normal colon mucosa. Furthermore, both enzymatic activities in carcinoma decreased proportionally, supporting the concept that mitochondrial respiration in carcinoma and normal tissue are quantitatively but no qualitatively changed (60).

The decrease in the mitochondrial enzyme activities of complex I (NADH-ubiquinone reductase) and of complex IV (cytochrome oxidase) in the non-tumor adjacent colon mucosa are to be considered as an indication of mitochondrial dysfunction that contributes to adjacent cell apoptosis and tumor progression.

Table 2. Mitochondrial enzyme activities in submitochondral membranes of tumor and non-tumor adjacent tissue from patient in initial or advanced stages of evolution of human colorectal carcinoma

	Initial stage		Advanced stage	
	Non-tumor	**Tumor**	**Non-tumor**	**Tumor**
NADH-cytochrome c reductase (complex I + III)[a]	694 ± 60	644 ± 60	481 ± 37[1]	355 ± 30[1 2]
Succiante-cytochrome c reducatse (complex II +III)[a]	159 ± 15	161 ± 15	160 ± 15	160 ± 15
Cytochrome oxidase (complex IV)[a]	228 ± 21	238 ±20	142 ± 17[1]	94 ± 11[1 2]
mtNOS[b]	3.5 ± 0.5	5.4 ± 0.7	7.6 ± 1[1]	7.9 ± 1[1]*

The activities of complexes I-III, II-III, and IV were determined spectrophotometrically (58,64). Mitochondrial NO production (mtNOS activity) was determined by the oxyhemoglobin (HbO_2) oxidation assay as described (81). Units: [a] nmol substrate / min. mg protein; and [b] nmol NO /min . mg protein. Values are means ± S.E.M. Differences between groups were analyzed by the Student-Newman-Keuls post hoc test after significant one way ANOVA. [1] $p < 0.05$ for advanced stage *v.s.* initial stage.; [2] $p < 0.05$ for tumor *v.s.* non-tumor.

7. SUPEROXIDE DISMUTASE ACTIVITIES IN HUMAN COLORECTAL CANCER TISSUE

The levels of superoxide dismutase (SOD) activity in cancer cells, considering both cytosolic Cu,Zn-SOD and mitochondrial Mn-SOD, have been extensively studied as an approach to determine oxyradical metabolism and turnover in cancer cells (61-63). Reports on SOD activity in colon cancer exhibit deep discrepancies, likely derived from the different methods used to determine the enzyme activities (64). In colorectal cancer, total SOD activity was reported significantly increased (41-43) and significantly decreased (44,65). In our study, we measured Cu,Zn-SOD and Mn-SOD activities in non-tumor and tumor tissues in the initial and advances stages of human colorectal cancer (Figure 1). Both activities were higher in tumor than in non-tumor tisues in both stages: tumor tissue showed 1.5-2.0 times more Cu,Zn-SOD activity and 1.5-1.4 times Mn-SOD activity, in initial and advanced stages, respectively. Interestingly, tumor progression shows decreased (42%) Cu,Zn-SOD activity and unchanged Mn-SOD activity.

There is an active interest in the clinico-pathological significance of immunohistochemical Mn-SOD expression in colorectal carcinoma. Lymph node metastasis was higher in Mn-SOD positive carcinomas than in Mn-SOD negative carcinomas. Survival in patients with Mn-SOD positive carcinomas was significantly lower than in patients with Mn-SOD negative carcinomas (66).

8. NITRIC OXIDE AND HUMAN COLORECTAL CANCER PROGRESSION

In the digestive tract, NO is widely distributed from the mouth to the anus. It is involved in splanchnic and systemic hemodynamics, in mucosal protection, in immune mechanisms, in hepatic function and in endocrine secretion. It is also involved in peristalsis as an inhibitory non-adrenergic and non-cholinergic neurotransmitter and relaxant of the smooth musculature (67).

Nitric oxide is starting to be considered as a pleiotrophic regulator, pivotal to numerous biological processes, including carcinogenesis. The role of reactive nitrogen species in colon carcinogenesis seems multifactorial and affecting a series of processes, such as proliferation, apoptosis, differentiation, tumorogenesis, and metastasis. Over-expression of inducible nitric oxide sinthase (iNOS) is a common phenomenon during chronic inflammatory conditions and has been well established in carcinogenesis with specific reports of increased iNOS expression in colon tumors (68,69). Progression of a large number of human and experimental colon tumors appears mediated by NO and inflammatory cytokines with inactivation by nitrosylation of p53-mediated caspase activity in the tumors. In some cases, NO-mediated induction of apoptosis was associated with tumor regression (70). This dichotomy is largely explained by the complexity of the signaling pathways in tumor cells, that respond differently to NO depending on NO concentration, on normal or mutated p53, mutation, and on activation or inactivation of apoptotic proteins. Evidence from both *in vitro* and *in vivo* support that the idea that NO and its reactive metabolite peroxynitrite stimulate cyclooxygenase-2 (COX-2) leading to generation of prostaglandins and to tumor growth (71). Thus, a NO-mediated signaling including activation of COX-2 is likely involved in the regulation of tumor growth and metastasis by promoting invasion and angiogenesis (40,70). A higher expression of iNOS in colorectal cancer cells was observed in the initial Dukes stages A and B as compared with the advanced Dukes stages C and D (72).

The role of mtNOS and mitochondrial NO production in carcinogenesis and cancer progression is an open question. It is probable that mitochondrial NO is involved in the regulation of O_2 uptake, cell signalling and tumor progression. We have recently observed substantial changes in mtNOS activity in cell proliferation and apoptosis in an over-stimulated rat ovarian cycle (73). It seems that relatively low levels of NO drive cell signaling for follicle proliferation, whereas relatively high NO levels trigger mitochondria-dependent follicle apoptosis. The observed increase of mtNOS activity in the ovary proliferative phase was associated with an increase in mitochondrial mass, understood as an augmented mitochondrial biogenesis during cell proliferation. In human colorectal cancer tissue mtNOS activity was relatively low at the initial stage of tumors and clearly increased (46%) in the advanced stage. Again, the adjacent non-tumor tissue showed a similar increased in mtNOS activity along cancer progression (Table 2). A positive linear correlation between mtNOS activity and mitochondrial oxidative damage was observed when these two variables were considered in the four experimental groups (Figure 2).

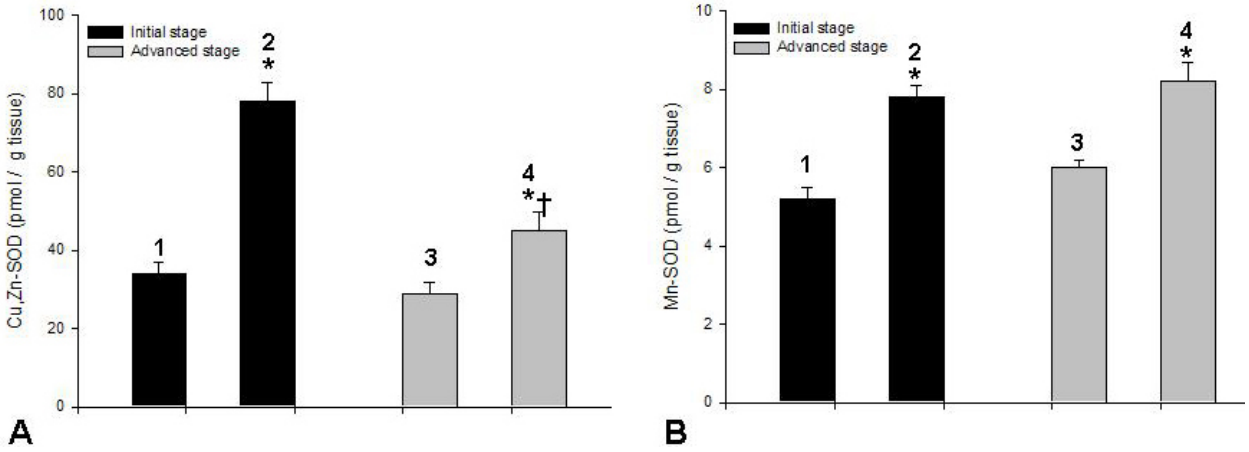

Figure 1. Cytosolic (Cu,Zn-SOD) and mitochondrial (Mn-SOD) superoxide dismutase activities in non-tumor and tumor tissues from human colorectal cancer in different stages of progression. (A) Cu,Zn-SOD activity: (1) non-tumor tissue of human colorectal carcinoma in initial stage; (2) tumor tissue of human colorectal carcinoma in initial stage. (3) non-tumor tissue of human colorectal carcinoma in advanced stage; (4) tumor tissue of human colorectal carcinoma in advanced stage (B) Mn-SOD activity: (1) non-tumor tissue of human colorectal carcinoma in initial stage; (2) tumor tissue of human colorectal carcinoma in initial stage. (3) non-tumor tissue of human colorectal carcinoma in advanced stage; (4) tumor tissue of human colorectal carcinoma in advanced stage. Mitochondrial (Mn-SOD) and cytosolic (Cu,Zn-SOD) superoxide dismutase activities were determined in tissue homogenates by the adrenochrome spectrophotometric assay followed at 480 nm ($E = 4.0\ mM^{-1}\ cm^{-1}$) in a reaction medium containing 1 mM epinephrine, 1 mM KCN, and 50 mM glycine/KOH (pH 10.0) (64,82,83). Values in pmol / g tissue are means ± S.E.M. Differences between groups were analyzed by the Student-Newman-Keuls post hoc test after significant one way ANOVA. [1] $p < 0.05$ for tumor *v.s.* non-tumor; [2] $p < 0.05$ for advanced stage *v.s.* initial stage.

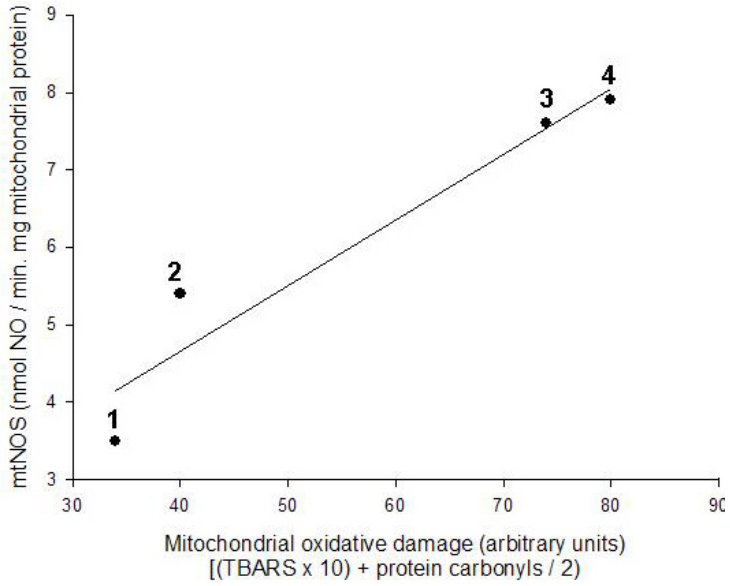

Figure 2. Correlation between mtNOS activity and mitochondrial oxidative damage in human colorectal cancer. (1) Non-tumor tissue of human colorectal carcinoma in initial stage; (2) tumor tissue of human colorectal carcinoma in initial stage; (3) non-tumor tissue of human colorectal carcinoma in advanced stage; and (4) tumor tissue of human colorectal carcinoma in advanced stage. $r^2 = 0.92$, $p < 0.01$.

Our results agree with an *in vitro* study of the mtNOS modulation by cellular redox state in M3, MM3, and P07 murine tumors and their respective cell lines, that showed that low mitochondrial NO-dependent H_2O_2 may be a platform to explain persistent tumoral growth (74).

The fine regulation by H_2O_2 of the physiological cell cycle was advanced by Antunes and Cadenas (75) that observed in Jurkat T-cells that H_2O_2 steady state concentrations below 0.7 microM place cells in a proliferative state, whereas at 1.0-3.0 microM H_2O_2 cells

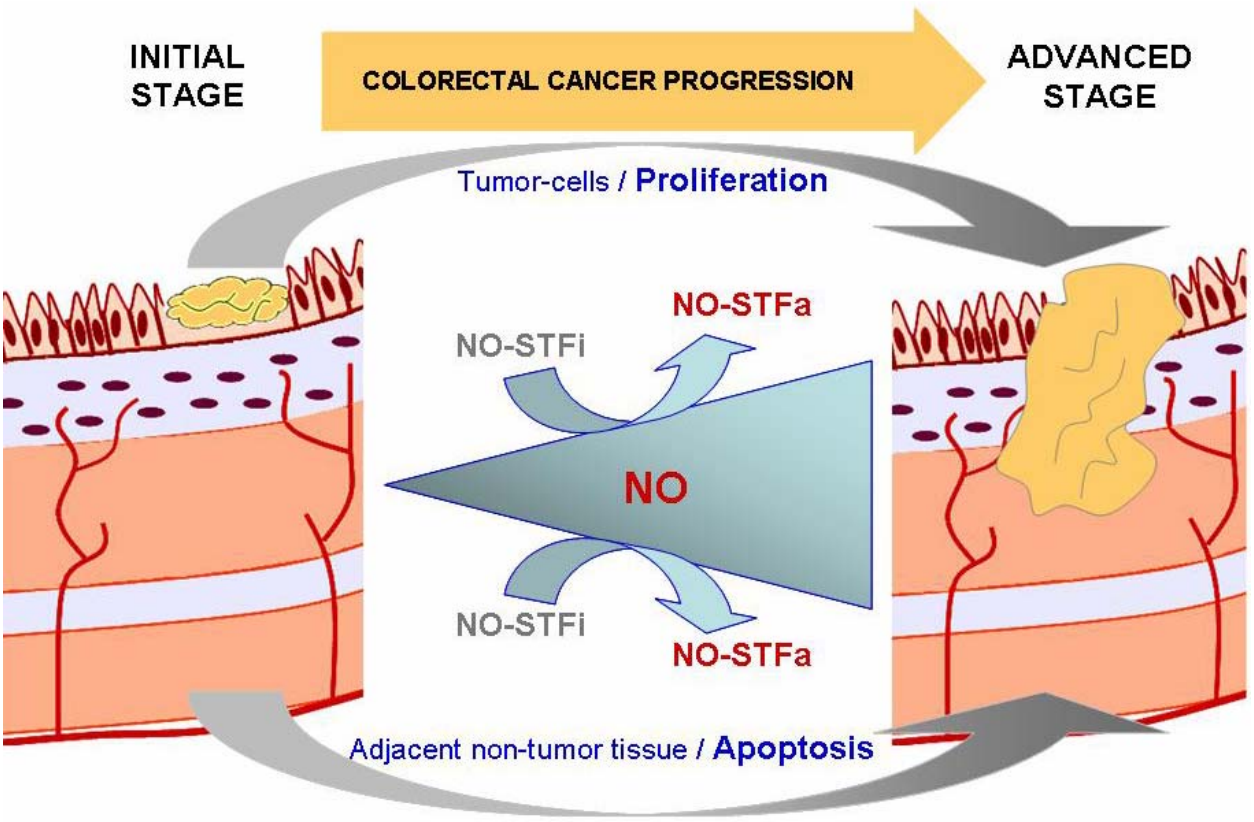

Figure 3. Mechanism of action of mitochondrial NO in colorectal tumor progression. The NO-sensitive inducible transcription factor (NO-STF) promotes tumor cell proliferation in tumor tissue and promotes apoptosis in adjacent non tumor-tissue. NO-STFi: NO-sensitive inducible transcription factor in inactive form, and NO-STFa: NO-sensitive inducible transcription factor in active form.

develop apoptosis, and that at levels higher than 3.0 microM H_2O_2 cells undergo necrosis.

Changes in oxygenation of tissues are transduced to adequate intracellular adaptive responses mediated by hypoxia-inducible factor-1 (HIF-1). Activation of HIF-1 provokes pro-survival as well as pro-death decisions under hypoxia. It became apparent that hypoxia can initiate cell demise by apoptosis/necrosis but also prevent cell death by provoking adaptive responses that, in turn, facilitate cell proliferation or angiogenesis, thus contributing to tumor progression (76). Mitochondrial NO could induces a NO-sensitive inducible transcription factor (NO-STF) that promotes tumor cell proliferation in tumor tissue and apoptosis in adjacent non tumor-tissue, facilitating colorectal cancer progression (Figure 3). The proposed mechanism of action of the nitric oxide in tumor progression is similar to the induction of peroxisome proliferator-activated receptor gamma coactivator 1alpha in mitochondrial biogenesis (77)

9. PERSPECTIVE

Human colorectal cancer progression was found associated to increased oxidative damage, decreased activity of mitochondrial electron transfer complexes and to increased mtNOS activity. The direct relationship between oxidative damage and mtNOS activity suggest a role of mitochondrial NO and H_2O_2 in tumor progression. It seems that the fine regulation of mitochondrial NO and H_2O_2 levels can drive the tumor and adjacent non-tumor cell to proliferation or to apoptosis, depending on the steady state concentrations and production kinetics. Relatively low levels of NO and H_2O_2 seem to drive the cell signaling for tumor progression, whereas relatively high NO levels might trigger mitochondria-dependent tumor apoptosis. The relation between mtNOS activity and oxidative damage supports the concept that mitochondrial NO and mitochondrial H_2O_2 diffuse from tumor tissue to adjacent non-tumor tissue, mediating an oxidative damage and signaling to non-tumor neighbor tissue as the classic toxohormones.

10. ACKNOWLEDGMENTS

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