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REVIEW ARTICLE

Protein–polyamine conjugates by transglutaminase 2 as potential markers for antineoplastic screening of natural compounds

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Abstract The role of post-translational modification of cell proteins with polyamines, a reaction catalyzed by a tissue tranglutaminase (TG, EC 2.3.2.13), in the induction of cell differentiation, represents an intriguing strategy to control cell proliferation and metastatic ability of different tumor cell lines. In this review, we focus our attention on the metabolic aspects of some natural compounds (methylxantines, retinoids and flavonoids) responsible of their antitumor effects exerted through the induction of TG activity in cancer cells.

Keywords Polyamines · Transglutaminase · Melanoma · Differentiation

Introduction

Polyamines are naturally occurring polycationic alkylamines absolutely required for eukaryotic cell growth. They possess specific roles in embryonic development (Kusunoki and Yasumasu 1978), cell cycle (Seidenfeld et al. 1981), immune system (Seiler and Atanassov 1994), neurological (Seiler 2000) as well as pulmonary functions (Hoet and Nemery 2000). Their intracellular levels are regulated by multiple pathways, such as neosynthesis from aminoacid precursors, cellular uptake mechanisms, stepwise degradation and efflux (Thomas and Thomas 2001).

Interestingly, the cytoplasmic content of polyamines, as well as their metabolic pathway, are frequently unregulated in cancer cells, providing a unique set of targets for therapeutic intervention (Seiler 2003). In fact, polyamine biosynthesis in tumor cells is highly upregulated, and this evidence has been correlated with the increased tumor cell proliferation rate, with respect to the normal counterpart. To this matter, the intracellular content of polyamine has been proved to be an important marker of cell proliferation (Bachrach et al. 2001).

The elucidation of the role of polyamines remains a challenging problem. Their involvement in the control of events as important as cell proliferation and differentiation is intriguing. Although a simple mechanism of action seems difficult to reconcile with the multiplicity of effects, recent investigations have narrowed the previously proposed hypothesis to a few possibilities. One of the most plausible is the regulation of the function of at least some proteins through their post-translational modification (Cordella-Miele et al. 1993). In this sense, the structural requirements for polyamine activity in various organs and cell cultures as modifiers of proteins imply that a common fundamental mechanism underlies their effects. (Beninati et al. 1988). Recently, a direct correlation between the formation of protein–polyamine conjugates and the increase of TG activity has been shown. We have been particularly interested in natural agents usually present in the human diet, that suppress cell growth inducing differentiation involving the post-translational modification of proteins with polyamines.

Transglutaminases and polyamines

The involvement of a TG, namely the catalytic domain of factor XIII, in blood clotting in vertebrates has triggered a number of functional studies of these enzymes. To date, several functional types of TGs, which differ in specificity

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toward target proteins, have been characterized in humans (Aeschlimann et al. 1995). They are involved in a variety of protein modifications associated with animal development and pathology (Muszbek et al. 1996). One of them, band 4.2 protein, has lost its enzymatic activity and plays a structural role as a cytoskeleton component (Cohen et al. 1993).

Genes for members of the TG family typified by factor XIII have been characterized in a wide range of vertebrates and invertebrates (Cariello et al. 1997). TG activity has been detected also in plants but the respective genes so far have not been cloned (Serafini-Fracassini et al. 1995). In contrast to their limited glutamine substrate specificity, TGs possess an exceptionally wide specificity for amine substrates. This possibility provided the basis for some speculation concerning the role of TG and polyamines as modifiers of cancer cell protein in metastasis. Moreover, TG is expressed as part of the host response to the metastatic spread of tumors and also limits the tumor growth by initiating a fibrotic response against it. Also, tumor cells transfected with TG reduce their growth and metastatic ability, prolonging the survival of the tumor-injected mice.

In contrast to the other members of this protein family, tissue TG (tTG) is a multifunctional enzyme apparently involved in very disparate biological processes. By virtue of its reciprocal Ca^{2+}-dependent cross-linking activity, or GTP-dependent signal transducing activity, tTG exhibits multifunctionality at the molecular level. The cross-linking activity can subserve disparate biological phenomena, depending on the location of the target proteins. Intracellular activation of tTG can give rise to cross-linked protein envelopes in apoptotic cells, whereas extracellular activation contributes to stabilization of the extracellular matrix (ECM) and promotes cell–substrate interaction (Beninati et al. 1994). While tTG synthesis and activation is normally part of a protective cellular-response contributing to tissue homeostasis, the enzyme has also been implicated in a number of pathological conditions including fibrosis, atherosclerosis, neurodegenerative diseases, celiac disease, and cancer metastasis.

To appreciate the potential role of transglutaminases (TGs) in polyamine metabolism, it is necessary to briefly describe the reaction catalyzed by these enzymes. The TG-catalyzed reaction of a protein-bound polyamine [N-*mono*(γ-glutamyl)polyamine] with a second glutamine residue may lead to a covalent cross-link between two polypeptide chains by a N,N-*bis*(γ-glutamyl)polyamine bond (Folk 1980). In fact, TGs catalyze an acyl transfer reaction where the γ-carboxamide group of a peptide-bound glutamine is the acyl donor. Primary amino groups of many low molecular weight amines may act as acyl acceptors with the formation of *mono*-substituted γ-carboxamides of peptide-bound glutamic acid. Alternatively, the primary amine group may derive from the ε-portion of a lysine, leading to an ε-(γ-glutamyl)lysine isopeptide bond between endo-γ-glutaminyl and endo-ε-lysil residues in polypeptides. The latter reaction results in the formation of inter- or intramolecular covalent cross-links.

The finding of high levels of polyamines associated with the acid–insoluble protein fractions of rat liver, kidney and testis, provided impetus for a carefully conducted investigation on the nature of this protein–polyamine association, (Beninati et al. 1985). The biological significance of this covalent protein modification was highlighted in terminal differentiation of human keratinocytes leading to cornified cell envelope formation in skin (Martinet et al. 1990). Extended studies pointed out that the amount of *mono*- or *bis*-(γ-glutamyl)polyamines is dependent on free polyamine intracellular levels, suggesting that the presence of protein–polyamine crosslinks may be strongly affected by proliferative and/or differentiative stimuli (Folk et al. 1980).

Polyamine incorporation into cancer cell protein

Previous investigations suggest that the posttranslational modification of protein with polyamines may affect metastasis formation. We provided evidences for the formation and intracellular localization of γ-glutamyl-polyamine derivatives in two murine melanoma cell lines with different metastatic potential (Beninati et al. 1993b). Although the results demonstrated the presence of protein-bound polyamines in these cancer cells, pronounced differences were observed in the two cell lines investigated. Whereas few polyamine conjugates were found in highly metastatic B16-F10 cells, many of those were identified in the lowly metastatic counterparts, B16-$F10^{Lr6}$. The finding of N^1,N^8-bis(γ-glutamyl)spermidine in the proteolytic digest from the less metastatic cell line (B16-$F10^{Lr6}$), suggests a role for this cross-link in the modulation of the metastatic potential of melanoma cells. Commonly, TG activity in tumor cells is quite low and the levels of free polyamines is higher compared to the normal counterpart. Therefore, the possibility of increasing intracellular TG activity and consequently the amount of protein–polyamine conjugates has been considered a promising approach for cancer research. The role of the posttranslational modification of ECM and basement membrane (BM) proteins with polyamines in the metastatic process, catalyzed by an activated TG, has been extensively investigated in B16-F10 murine melanoma cells (Lentini et al. 2008).

Cell differentiation and cancer prevention

Cancer prevention by dietary factors or other agents is likely to represent one of the strategies to reduce the risk of

development of malignancy in humans. Carcinogenesis develops in three defined stages (initiation, promotion, and progression), the first and third resulting from irreversible genetic changes, whereas the intermediate stage of promotion involves an epigenetic alteration of the expression of the genome and cell division. Thus, the stage of promotion is the most-effective site to target for cancer prevention. A review of the modalities presently used in cancer prevention, demonstrates that all such methods involve inhibition and/or reversal of the promotion stage, thus preventing the development of the malignant process. Future advances in cancer prevention will depend on better knowledge of the mechanisms of the stages of carcinogenesis, in the light of the use of rational preventive agents able to alter the appropriate stage(s) of neoplastic development. There may be a role for differentiation therapy as a chemopreventive strategy, whereby patients at risk for the development of malignancy could take differentiation agents as prophylaxis against the occurrence of cancer. By and large, most of the differentiation agents studied to date have demonstrated significantly less toxicity as compared to standard cancer treatments. Because of its low toxicity profile, differentiation therapy could be employed effectively in cancer prevention in selected circumstances. Indeed, differentiation therapy is a new approach to the treatment of advanced or aggressive malignancies and showed significant efficacy in the treatment of many types of cancer (Jimenez and Yunis 1987). The application of this therapy halts the progression of the cancer, allowing the transformed cells to regain the morphology and cell functions of a mature cell, much like a cell from the organ where the cancer cell originated. While this would not eradicate the cancer, it would stop the growth of the tumor and halt metastatic progression, allowing the application of more conventional therapies to eradicate any cancerous growths (Leszczyniecka et al. 2001).

Several research projects are focused on different approaches to the study of cancer cell differentiation. Many of them, involve the study of the overall effect of novel differentiation agents on cultured cancer cells. Specifically, the effects of these agents on cancer cell growth, on changes in cellular morphology, and eventual stimulation to tumor cell differentiation and/or programmed cell death. The results of these experimental trials could prove beneficial for identifying molecules and pathways important in cancer progression and for increasing the efficacy of chemoprevention, providing the logistics for the application of new drugs as differentiation agents in patients at risk for the development of malignancy.

Our working interest in this field is focused on the identification of new molecules able to increase TG activity in cultured human and murine cancer cell lines with the aim to reduce their proliferation and invasive potential through the induction of cell differentiation (Beninati et al. 1993b).

Differentiation inducers related to TG

Methylxanthines

Methylxanthines (MXs) are natural compounds of interest because of the widespread ingestion of MX-containing beverages, such as coffee and tea. In addition, some MX are used as therapeutic agents. Among these alkaloids, the best known are theophylline (1,3 dimethylxanthine), theobromine (3,7 dimethylxanthine) and caffeine (1,3,7 trimethylxanthine). It has been shown that MXs act as cAMP-phosphodiesterase inhibitors (Bergstrand 1980), involving alterations in cAMP system tumor cell. Many of the biological effects of adenosine can be reverted by MXs, suggesting a role as adenosine antagonists (Fredholm 1980). In fact, adenosine stimulates the formation of cAMP and the adenosine-receptor-mediated actions are antagonized by MXs. There are at least two types of adenosine receptors on cells, A1 and A2. These two receptors are associated to a plasmamembrane-bound adenylate cyclase (AC) through G proteins. A1-receptors act through a Gi protein, which inhibits AC, while the A2-receptors function through a Gs protein, which stimulates AC. MXs possess a higher affinity for the A1-receptors, thus leading to an overproduction of cAMP (Fig. 1).

Several MXs have been found to induce differentiation and to inhibit the growth of murine melanoma cells to varying degrees, and the most potent among them were theophylline and caffeine (Lentini et al. 1997). Among

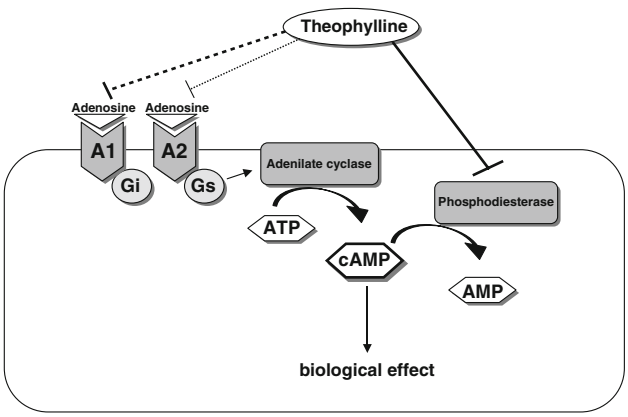

Fig. 1 Mechanism of action of theophylline. The molecule inhibits phosphodiesterase and antagonizes adenosine for the binding to A1 and A2 receptors. Due to the higher affinity for the A1 receptor (associated to inhibitor G protein, Gi), the production of cAMP mediated by A2 receptor, stimulatory G protein (Gs) and adenylate cyclase, is enhanced

MXs, theophylline has been shown to induce apoptosis in a variety of tumor cell lines, including murine B16-F10 melanoma cells (Lentini et al. 2000a), human melanoma MEL-120 (Facchiano et al. 2001) and several epithelial cancer cells (Caraglia et al. 2002). Clinical studies of theophylline use in cancer therapy is limited to a case report published on the therapeutic efficacy of this MX in chronic lymphocytic leukemia (Makower et al. 1999).

The correlation between metastatic power of cancer cells and the posttranslational modification of protein catalyzed by TGs, appears supported by our recent findings on murine B16-F10 melanoma cells treated with MXs. We reported that theophylline is able to enhance TG activity in cancer cells, to induce melanoma differentiation markers (Table 1), and to increase intracellular concentration of protein–polyamine cross-links (in particular N^1,N^8-*bis*-[γ-glutamyl]spermidine) to levels comparable to the lowly metastatic counterparts B16-F10^{Lr6} (Beninati et al. 1993b). The in vivo capacity of this MX to influence the metastatic behavior of B16-F10 melanoma cells was investigated by injecting theophylline-treated melanoma cells into the bloodstream of syngeneic mice (C57BL6/N). This treatment impaired the ability of theophylline-treated cells to invade the target organ, lowering the frequency of metastatic foci by about 70% (Fig. 2), and increasing the lifespan of the tumor-injected animals, compared to controls (Lentini et al. 1998; 2000a). In order to evaluate and discriminate between the in vivo antiproliferative and antiinvasive properties of MXs, we published a new procedure based on the integrated image analysis performed on the histological sections of the target organ (Lentini et al. 2000b). The data obtained by this procedure suggest that theophylline, and partially caffeine, reduced the frequency of tumor metastases and their growth, thus sharing two dominant effects on B16 melanoma cells, antimetastatic and antiproliferative (Table 2).

Since adhesion and migration through the ECM and BM represent main steps of the metastatic cascade, we focused our attention on the effect of the TG-mediated posttranslational modification of ECM proteins with polyamines. Alterations in the size and structure of the ECM protein side chains by TG and polyamines secreted by tumor cells are now considered crucial in the impairment of adhesiveness and metastatic properties of cancer cells. We collected recent evidences on the posttranslational modification of some BM proteins (laminin, fibronectin, Matrigel) with polyamines as a possible mechanism responsible for the control of B16-F10 melanoma cell metastaticity. In fact, the TG-mediated modification with

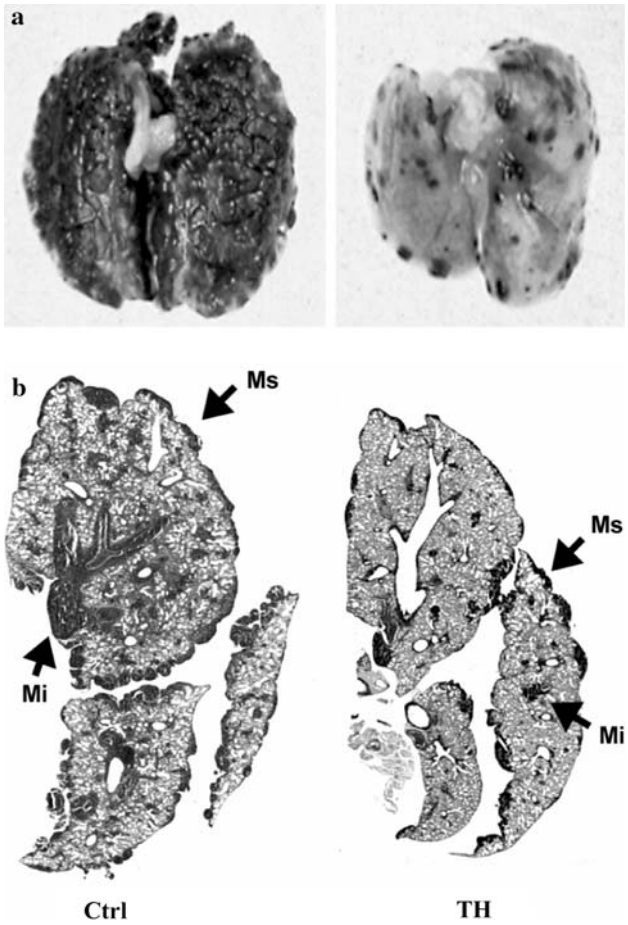

Fig. 2 Representative lungs (**a**) and histological sections of lung (**b**) colonized by B16-F10 melanoma cells untreated (Ctrl) or treated with 1 mM theophylline (TH). *Ms* superficial metastases, *Mi* internal metastases

Table 1 Effect of theophylline (TH) treatment of B16-F10 melanoma cells on TG activity, melanin content and tyrosinase activity as differentiation markers

B16-F10 cell treatment	TG activity (pmol [14C]-put/mg prot)	Melanin content (μg/mg prot)	Tyrosinase activity (cpm/h per mg prot)
–	6.0 ± 1.2	5.0 ± 0.7	1,000 ± 120
0.1 mM TH	9.0 ± 0.8[a]	9.2 ± 0.9[b]	1,700 ± 150[b]
1 mM TH	120.0 ± 10.7[b]	25.5 ± 3.2[b]	2,560 ± 350[b]

From: Beninati et al. (1993b) Int J Cancer 53:792–797

[a] Significantly different from control, $P < 0.005$

[b] Significantly different from control, $P < 0.001$

Table 2 Morphometric parameters obtained by computer-assisted image analysis performed on histological sections of B16-F10 invaded mice lungs and their correlation with a growth (TG activity) and invasion (hydroxyproline levels) markers

B16-F10 cell treatment	Growth index (GI) ($\times 10^4$)	Correlation with TG activity (r)	Invasion index (II)	Correlation with hydroxyproline levels (r)
–	160 ± 10	−0.903 ($n = 10$; $P < 0.01$)	369.7 ± 21.7	0.948 ($n = 10$; $P < 0.01$)
1 mM theophylline	48 ± 3[a]	−0.876 ($n = 10$; $P < 0.01$)	105.8 ± 17.7[a]	0.919 ($n = 10$; $P < 0.01$)
1 mM caffeine	62 ± 5[a]	−0.885 ($n = 10$; $P < 0.01$)	278.2 ± 16.4[b]	0.650 ($n = 10$; $P < 0.05$)
1 mM theobromine	156 ± 12	−0.959 ($n = 10$; $P < 0.01$)	389.1 ± 22.3	0.959 ($n = 10$; $P < 0.01$)

From: Lentini et al. (2000b) Eur J Cancer 36(12):1572–1577

r Bravais-Pearson correlation coefficient

[a] Significantly different from control, $P < 0.001$

[b] Significantly different from control, $P < 0.005$

polyamines of these proteins strongly decreased melanoma cell adhesion and their in vitro invasive activities (Lentini et al. 2008).

In summary, the role of MXs as TG activators and differentiative molecules, triggering the production of protein-polyamine conjugates, becomes of particular interest in the field of cancer research.

Retinoids

Retinoids are naturally occurring and synthetic analogs of vitamin A (retinol), which regulates a variety of physiologic processes including growth, vision, reproduction, epithelial cell differentiation, and immune function. The primary dietary sources for retinol are carotenoids from vegetables and retinyl esters from animal tissues. Retinol plays an important role in visual response, while most of the other functions of retinoids are thought to be mediated by its oxidized product, *all-trans* retinoic acid (ATRA). ATRA is derived from the intracellular oxidation of preformed retinol absorbed from the gastrointestinal tract.

ATRA enters the cell by simple diffusion or by conversion from retinol absorbed in the intestine, bound in circulating form to retinol-binding proteins (RBP), and rebound intracellularly to cellular retinol-binding proteins (CRBP) (Fig. 3). ATRA can be metabolized immediately after binding to cellular ATRA-binding proteins (CRABP) and oxidized by cytochrome P450 enzymes located in smooth endoplasmic reticulum. Alternatively, ATRA or its isomers enter the cell nucleus and bind to ATRA receptors (RAR) or to retinoid "X" receptors (RXR). After dimerization (ie, the formation of an RAR/RXR heterodimer or an RXR/RXR homodimer), these reactivated receptors bind with high affinity to specific DNA segments (the ATRA response element) and induce transcription of their mRNA. Ultimately, the retinoid response is mediated by primary target genes, by interference with other transcription factors, and by control of certain posttranscriptional actions (Goodman et al. 1993). ATRA is the prototype of a differentiation therapy agent. It was the first differentiation agent found to be successful in the treatment of acute promyelocytic leukemia (APL) (Tallman et al. 2002), which is characterized by an abnormal proliferation of promyelocytes. Most APL patients are now treated first with ATRA, which induces a complete remission in about 70% of cases. Interestingly, the stimulation of differentiation in leukemia has been linked to the induction of the apoptotic process. As example, leukemic cells exposed to differentiation inducers ultimately die an apoptotic death, generally as a rather late event (Martin et al. 1990). This phenomenon is accompanied by an increase in TG expression, providing additional support for a role of these enzymes in growth arrest and differentiation in cancer cells. Addition of ATRA to HL-60 human myeloid leukemia cells results in a dramatic increase in tTG activity. This ATRA-induced expression of tTG is potentiated by analogs of cAMP. A 50-fold increase of the enzyme activity can be detected within 6 h by the addition of the retinoid to cultured cells. The ATRA-mediated induction of tTG is a specific response of HL-60 cells, and is not seen when using other agents that induce HL-60 differentiation. The enhancement of tTG activity has been proposed as a marker of the early events in retinoid-regulated gene expression in both normal and transformed cells (Davies et al. 1985). Whereas treatment of the HL60 with ATRA results in cellular differentiation, addition of the synthetic retinoid, N-(4-hydroxyphenyl)retinamide (HPR), induces HL60 cells to undergo apoptosis. Moreover, pretreatment of HL60 cells as well as other cell lines (i.e. NIH3T3 cells) with ATRA blocks HPR-induced cell death. It has been observed that monodansyl-cadaverine (MDC), which binds to TG, eliminated ATRA protection against cell death and in fact caused ATRA to become an apoptotic factor, suggesting that the ability of ATRA to protect against apoptosis is linked to the expression of active TG (Antonyak et al. 2001).

Other considerable evidences on the effects of ATRA involve the growth of murine melanoma cells. Several

studies on the proliferation of the murine melanoma S91 and BL6 cell lines revealed that cell-growth arrest by retinoids is time- and dose-dependent and reversible (Lotan 1980). We have also found that ATRA induces tTG activity in both B16-F10 and B16-F10^{Lr6} melanoma cells (Beninati et al. 1993a). The increase in the soluble enzyme activity was directly related to the levels of polyamine covalently incorporated into intracellular proteins and inversely related to the metastatic potential of cancer cells. This apparent relationship again suggested a role for the conjugation of polyamines to cancer cell proteins in the metastatic process. Interestingly, there was a dichotomy of response in the two cell lines with differentiative changes only being evident in the more malignant (B16-F10) and aggressive cell line (Fig. 4). Since cAMP-dependent mechanisms play an important role in the establishment of experimental metastasis and highly metastatic melanoma clones possess an aberrant regulate adenylate cyclase (Lester et al. 1986), it is possible that the selectivity in tTG increase and polyamine incorporation observed for the highly metastatic cell line is due, at least in part, to the action exerted by cAMP on ATRA (Murtaugh et al. 1986).

Flavonoids

Flavonoids, widely distributed throughout the plant kingdom and abundant in flowers, fruits and leaves, are characterized by the presence of two aromatic rings linked by a 3-carbon bridge (to form chalcones) or by a pyrane or pyrone ring. The thousands of flavonoids known can be

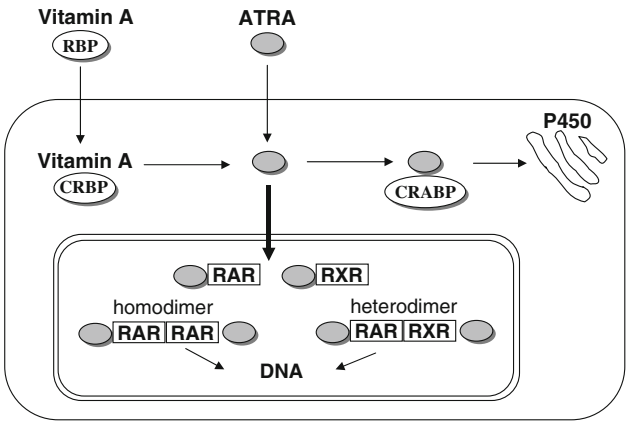

Fig. 3 Mechanism of action of retinoic acid (ATRA). ATRA enters the cell by simple diffusion. Alternatively, retinol (Vitamin A) from diet, bound to retinol-binding proteins (RBP), uptaken, rebound intracellularly to cellular retinol-binding proteins (CRBP) and is converted to ATRA. After binding to cellular ATRA-binding proteins (CRABP), ATRA is oxidized by cytochrome P450 enzymes located in smooth endoplasmic reticulum or enters the nucleus and bind to ATRA receptors (RAR) or to retinoid "X" receptors (RXR). After dimerization, the receptors bind DNA segments inducing their transcription

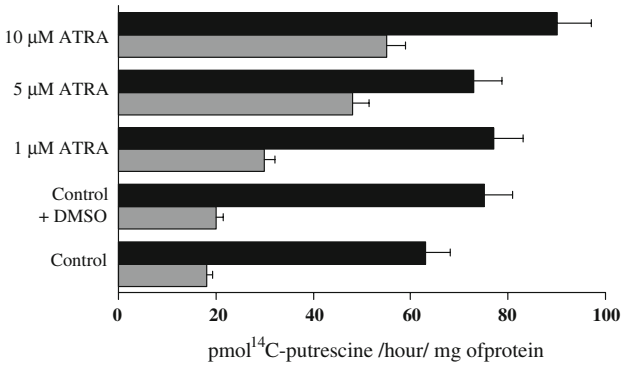

Fig. 4 Evaluation of tTG activity in ATRA-treated B16-F10 (*greyed square*) and B16-F10 Lr6 (*dark square*) cells for 48 h. ATRA was freshly dissolved in dimethylsulfoxide (DMSO) and diluted into the growth medium immediately before the incubation. Data represent the mean ± SD of five experiments. Data represent the mean ± SD of three different determinations and differences were considered significant when $P < 0.001$ (*) (*t*-Student's test)

classified into main classes: flavonols, flavones, flavanols (cathechins), flavanones, isoflavones and anthocyanin pigments. Flavonoids and flavonoids-rich extracts were implicated as beneficial agents in a multitude of disease states, included cancer (Spencer et al. 2003; Go et al. 2003). Although the antioxidant effects of some flavonoids, due to their capability to scavenge free radicals, have attracted the interest of the researchers, there are effects beyond antioxidation that may be important in determining the anticancer activity of phytochemicals, such as effects on cell proliferation, polyamine synthesis and TG activity.

Previous studies underline that some flavonoids, such as epigallocatechin-3-gallate (EGCG) and curcumin, are able to increase TG activity in primary keratinocytes, promoting cell differentiation (Balasubramanian et al. 2005; Balasubramanian and Eckert 2007). Flavonoids exhibit antioxidant activity (Rakotoarison et al. 1997) and inhibit cAMP phosphodiesterase with consequent increase in cAMP levels (Nikaido et al. 1989; Nikaido et al. 1988).

The antitumor activities of these compounds is correlated to their capability to regulate the signaling trasduction pathways in cancer progression, such us NF-kB, activator protein 1 or mitogen-activated protein kinases (Fresco et al. 2006).

Among the various types of flavonoids associated with cancer prevention, the flavanones naringenin and hesperitin, found in grapefruit and oranges, are of particular interest (Liu 2004). Indeed, both in vitro and in vivo antineoplastic activity of these molecules, associated to the marked increase in TG activity, have been recently observed in the murine melanoma B16-F10 cell line (Lentini et al. 2007).

The wider distribution in plant kingdom, together with promising results in cancer investigation, suggest that

flavonoids may contribute to the preventive effect of a plant-based diet on neoplastic disease. However, the effects of flavonoids on cancer needs further epidemiological study, since at the moment the in vitro results cannot be extrapolated directly to humans.

Conclusion

Differentiation therapy of cancer was conceived from the observation that tumor cells can regain control of growth and differentiation in response to a number of natural and synthetic compounds (Prasad et al. 2001). Although, several markers have been identified on tumor cells, their casual relationship to neoplastic competence has not been characterized in sufficient detail to warrant their evaluation as novel pharmacological targets for the design of new differentiative agents. The current rationale for targeting cAMP and tTG as contributing factors in cancer competence is based on several interrelated correlations (Lentini et al. 2004).

In recognition of this information, and in search of a novel approach for cancer chemoprevention, we have focused our attention on the investigation of the function of tTG in neoplastic growth, examining the posttranslational modification of protein in which structural elements of polyamines are involved. Although, such reaction may affect marginally the cellular metabolism of polyamines, the function of the polyamine-modified protein is specifically perturbed (Beninati and Mukherjee 1992). It is still unclear, however, whether this protein modification is casually associated with the acquisition and expression of high metastatic capacity or alternatively is merely a secondary property that is not essential for the pathogenesis of metastatic disease. We have followed these criteria in analyzing methylxanthines, retinoids and flavonoids responsiveness of the B16-F10 melanoma cell line. Results also indicate that either theophylline or ATRA induce change in the rate of posttranslational modification of protein by polyamines in cells having different metastatic potential, likely because these drugs increase the activity of tTG and affect the intracellular levels of cAMP (Lester et al. 1986). While this result is based on one histological class of tumor, the most important implication of our observation is that it establishes a new way in the screening systems for detecting new molecules able to prevent neoplastic growth.

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