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Anticancer drugs and hyperthermia enhance cytotoxicity induced by polyamine enzymatic oxidation products

Review Article

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Summary. A correlation between regulation of cell proliferation and polyamine metabolism is described. The latter can enter protein synthesis through the modification of eukaryotic initiation factor 5A (eIF5A) and the formation of the peculiar amino acid hypusine. Specific inhibitors of hypusine formation induce apoptosis that can be potentiated by the combination with cytokines such as interferonα (IFNα) that itself decreases hypusine synthesis. We have also demonstrated that the concomitant treatment of cancer cells with IFNα and the protein synthesis inhibitor fusion protein TGFα/*Pseudomonas Aeruginosa* toxin synergize in inducing cancer cell growth inhibition. Another way used by polyamines to induce apoptosis is the generation of intracellular oxidative stress through the interaction with bovine serum amine oxidase (BSAO). This enzyme used simultaneously to spermine induces apoptosis, necrosis, inhibition of cell proliferation and inhibition of DNA and protein synthesis in several cell types. The enzymatic oxidation products of polyamine, H_2O_2 and aldehyde(s) cause these effects. We have recently found that the cytotoxicity of anti-cancer agents, either etoposide or docetaxel, in cancer cells is potentiated in the presence of BSAO/Spermine. In conclusion, polyamine metabolites could be useful in the design of new therapeutic strategies.

Keywords: Amine oxidase – Polyamines – Multidrug resistance – Eukaryotic initiation factor 5A – Docetaxel – IFNα

Abbreviations: AO, amine oxidase; BSA, bovine serum albumin; BSAO, bovine serum amine oxidase; CHO, chinese hamster ovary; cyclin/cdks, cyclin-dependent kinase; DFMO, 2-(difluoromethyl)ornithine; DTX, docetaxel; EGF-R, epidermal growth factor receptor; eIF5A, eukaryotic initiation factor 5A; HTC, hepatoma tissue culture; IFNα, interferon alpha; MDL 72527, N^1,N^4-bis(2,3-butadienyl)-1,4-butanediamine; MDR, multidrug resistance; ODC, ornithine decarboxylase; PAO, polyamine oxidase; PEG, poly ethylene glycol; Put, putrescine; ROS, reactive oxygen species; SAMDC, S-adenosylmethionine decarboxylase; SAO, serum amine oxidase; Spd, spermidine; Spm, spermine; SSAT, spermidine/spermine N^1-acetyltransferase; VP16, etoposide

Introduction

Polyamines are ubiquitous molecules with a strong positive relationship to cell growth and to cancer development (Cohen, 1998). The role of polyamines in cell growth regulation could be at least in part explained with the correlation existing between polyamine metabolism and translational factors, involved in protein synthesis machinery as eukaryotic initiation factor 5A (eIF5A) whose activity is modulated by a series of post-translational modifications, that culminate in the formation of the unusual amino acid hypusine (Park et al., 1993). The specific deoxyhypusine synthase inhibitor GC7 avoids hypusine formation and blocks eIF5A activity. We have found that GC7 synergizes with interferon alpha (IFNα) in inducing cell growth inhibition and apoptosis suggesting a critical role for eIF5A in the modulation of cell proliferation induced by IFNα in human epidermoid cancer cells (Caraglia et al., 2003a). Another way used by polyamine metabolism is the generation of H_2O_2 and aldehydes formed by bovine serum amine oxidase (BSAO) in the presence of spermine (Spm). These polyamines metabolites can induce cell death or inhibit cell proliferation in human cancer cells (Averill-Bates et al., 1993; Agostinelli et al., 2004, 2006a). Moreover, it was observed that toxic products generated by the oxidative deamination of Spm by BSAO were also able to overcome multidrug resistant

phenotype of human cancer cells. The finding and experimentation of new strategies to potentiate apoptosis of cancer cells are the key for the discovery of novel anticancer therapy.

Polyamines and cancer

Polyamines putrescine ($H_2N(CH_2)_4NH_2$), spermidine ($H_2N(CH_2)_3NH(CH_2)_4NH_2$), and spermine ($H_2N(CH_2)_3HN(CH_2)_4NH(CH_2)_3NH_2$) are organic cations that are derived from amino acids and are found in all organisms. Polyamine biosynthesis in mammalian cells, begins with the production of putrescine (Put) by the decarboxylation of the amino acid ornithine, by ornithine decarboxylase (ODC). Subsequent addition of an aminopropyl group to Put leads to the synthesis of spermidine (Spd) and further addition of another aminopropyl group leads to the formation of Spm (Russell, 1980; Pegg and McCann, 1982). Aminopropyl group is derived by the decarboxylation of S-adenosylmethionine through the action of S-adenosylmethionine decarboxylase (SAMDC). ODC and SAMDC are rate-limiting enzymes in polyamine biosynthesis. Addition of aminopropyl groups to Put and Spd is catalyzed by the enzymes Spd synthase and Spm synthase, respectively.

It is well established that polyamines have a great diversity of functions in cell biology from growth-related effects (Cohen, 1998) to radical scavenging (Ha et al., 1998). Tabor and collaborators demonstrated by genetic studies that polyamines are essential for optimal growth and viability in bacteria (Tabor et al., 1980) and yeast (Tabor et al., 1992). Cleveland and collaborators have recently extended this paradigm to mammals, with the observation that the gene that encodes ODC – the enzyme required for the first stage in polyamine synthesis – is essential in mice (Pendeville et al., 2001). The mechanism of this requirement seems to involve the suppression of apoptosis by ODC in the developing mouse embryo (Pendeville et al., 2001). Polyamines have been widely implicated in the growth and development of a range of mammalian tissues and in remodelling processes associated with tissue repair.

It has been known for many years that there are changes in both ODC and polyamine concentrations during the cell cycle. There is an early peak of ODC at G1-phase, followed by an increase in polyamine content, and a later, second, increase during G2-phase and prior to mitosis (Bettuzzi et al., 1999). Thus both polyamines and cyclin/cdks (cyclin-dependent kinase) show phased changes through the cell cycle, but the interaction between these two sets of regulatory molecules remains to be defined. One suggestion is that polyamines regulate cyclin degradation (Thomas and Thomas, 2001). Intracellular polyamine concentrations have been reported to regulate both the up- and down-regulation of important cellular checkpoints within the cell cycle, and this may, in part, explain why their concentrations are controlled throughout the cycle (Kramer et al., 2001). With such a strong positive relationship to cell growth, it is perhaps not surprising that there has been an increasing effort over the last three decades to link polyamine metabolism to cancer development and to attempt to use inhibitors of polyamine biosynthesis as antiproliferative agents (Wallace et al., 2003).

More and more evidence points at the involvement of the natural polyamines in gene expression (Yamakura and Shimoji, 1999), including genes encoding cytoskeletal proteins (Thomas and Kiang, 1987). Gene expressions have mainly been investigated following polyamine depletion. Studies on signalling pathway demonstrated that the polyamines affect several phosphorylation reactions, and the expression of nuclear transcription factors (Yamakura and Shimoji, 1999). Disregarding tyrosine kinase the preferential targets of Put and Spd are not identical: Put activates the expression of c-jun and c-fos, while Spd activates c-myc, and the ERKs (Yamakura and Shimoji, 1999). Present information does not allow conclusions concerning a direct role of Spm in gene regulation. Spm seems not participating directly in gene expression regulation. 2-(difluoromethyl)ornithine (DFMO) prevents the expression of c-myc and c-fos.

Interestingly, polyamine synthesis is down regulated as cells become senescent in many tissues in adults (Chang and Chen, 1988). Polyamines affect numerous processes in carcinogenesis. Increased polyamine levels are associated with increased cell proliferation, decreased apoptosis and increased expression of genes affecting tumour invasion and metastasis. Conversely, suppression of polyamine levels is associated with decreased cell growth, increased apoptosis and decreased expression of genes affecting tumour invasion and metastasis (Ignatenko et al., 2004).

Polyamines are often present at increased concentration in tumour cells and tissues, for example, breast and colon cancer. In addition, depletion of polyamines leads to inhibition of tumour growth. (Averill-Bates et al., 2005).

The high concentrations of polyamines found in cancer cells are the result of several changes in polyamine metabolism. The regulation of ODC, for example, is altered in some tumours, resulting in increased ODC expression.

Several studies have confirmed that an increase in ODC activity and the subsequent increase in intracellular polyamine concentrations is an early event in carcinogenesis (reviewed in Wallace et al., 2001). More recent studies have linked polyamines to cell death, particularly the cellular suicide known as apoptosis (Schipper et al., 2000). Thus it now appears that the polyamines are bivalent regulators of cellular function, promoting cell growth or cell death depending on other environmental signals. Under normal circumstances polyamine concentrations regulate their own biosynthesis and prevent overproduction. However, in extreme cases, high exogenous polyamine concentrations can lead to cell death (Brunton et al., 1991).

About 30 years ago, the inhibition of cell growth due to the selective depletion of Spd has been demonstrated. Spm is not supporting cell growth directly but serves as a precursor of Spd and has, in addition, other functions (Wang et al., 2004). The search for inhibitors of polyamine-related enzymes started with the aim to inhibit tumour growth. Selective inhibitors of the enzymes involved in polyamine biosynthesis did not result in practically useful anticancer drugs (Marton and Pegg, 1995; Seiler, 2003), but a selective inactivator of ODC, 2-(difluoromethyl)ornithine (DFMO) is presently developed as a cancer chemopreventive agent (Gerner and Meyskens, 2004).

This selective inactivator of ODC is known to diminish Put and Spd concentrations of a wide variety of cells, and cause cell cycle arrest, mostly in G1 (Bey et al., 1987; Ackermann et al., 2003). It allowed identifying Spd as the actual growth-promoting compound among the polyamines. Polyamine depletion by DFMO affects cell function at multiple sites. Among many other effects protein synthesis is impaired (Hölttä, 1985) and topoisomerase II, an enzyme necessary for normal cell proliferation, looses its functionality if polyamines are depleted (Berntsson and Oredsson, 1999).

Protein synthesis regulation by polyamines: the role of eIF5A

The role of polyamines in cell growth regulation could be at least in part explained with the correlation existing between polyamine metabolism and translational factors involved in protein synthesis machinery. One of the factors modified by polyamines is eIF5A (Caraglia et al., 2003a). The latter is peculiar because its activity is modulated by a series of post-translational modifications that culminates in the formation of the unusual amino acid hypusine. Hypusine [N^{ε}-(4-amino-2-hydroxybutyl)lysine] is formed by the transfer of the butylamine portion from Spd to the ε-amino group of a specific lysine residue of eIF5A precursor and by the subsequent hydroxylation at carbon 2 of the incoming 4-aminobutyl moiety (Park et al., 1993). eIF5A probably acts in the final stage of the initiation phase of protein synthesis by promoting the formation of the first peptide bond (Hershey, 1991). Hypusine plays a key role in the regulation of eIF5A function because its precursors, which do not contain hypusine do not have activity (Park et al., 1993). These biochemical correlates make eIF5A peculiar. In fact, only the hypusine-containing eIF5A form is active and, consequently, the dosage of intracellular hypusine content measures also the activity of eIF5A since hypusine is contained only in this factor. More recently a correlation has been found between the polyamine-dependent modification of eIF5A and the triggering of apoptosis in tumour cells (Abbruzzese et al., 1989; Caraglia et al., 2003a).

As the hypusine is essential for the proliferation of mammalian cells (Park et al., 1993), it is often difficult to judge whether Spd depletion-induced growth arrest is due to the polyamine itself or whether it is related to the reduction of hypusine to form a functional translation initiation factor (Janne et al., 2004).

Extensive studies to clarify the role of eIF5A and polyamines in cell proliferation have been undertaken using inhibitors of the polyamine biosynthetic enzymes. Inhibitors of *S*-adenosylmethionine decarboxylase, e.g. 5-([(Z)-4-amino-2-butenyl]methylamino)-5-deoxyadenosine (AbeAdo), caused the depletion of Spd and Spm with a compensatory increase in Put, leading to a delayed cytostasis in L1210 cells (Byers et al., 1992). This growth arrest of L1210 cells by AbeAdo was supposed to be attributed mainly to the deprivation of hypusine in eIF5A. Inhibition of ODC by DFMO caused an effective depletion of Put and Spd, and this was accompanied by the inhibition of cell proliferation in rat hepatoma tissue culture (HTC) cells (Mamont et al., 1978) and other mammalian cells (McConlogue and Coffino, 1983; Korhonen et al., 2001). Since only a very small portion of cellular Spd was used for hypusine synthesis, it was not clear whether the growth inhibition, in the case of DFMO treatment, was due to the depletion of hypusine-containing eIF5A or a decrease in total polyamines. It was reported that GC7 (*N1*-guanyl-1,7-diaminoheptane), a potent inhibitor of deoxyhypusine synthase, selectively inhibits the formation of active eIF5A (Jakus et al., 1993). The correlation between hypusine, and thus eIF5A activity,

and cell proliferation suggests that activated eIF5A might play a role in cell growth and differentiation (Abbruzzese, 1988).

Polyamine metabolism and their oxidation products: the role of BSAO

The addition of Spm in the drinking water of C57BL mice caused a marked increase in the size of untreated melanoma tumors, when compared to tumours of mice drinking regular water. This finding is consistent with the necessity of polyamines for tumour growth (Averill-Bates et al., 2005).

On the other hand, the addition of Spm to culture medium containing serum inhibits cellular proliferation. This effect is caused by the toxic products of polyamine oxidation that are generated by serum amine oxidase (SAO). BSAO is a copper-containing glycoprotein of 170 kDa, which oxidatively deaminates the primary amino groups of polyamines, such as Spm and Spd. The reaction involves oxygen and water as substrates. The products are H_2O_2, aldehydes and ammonia. In the case of Spm, the monoaldehyde, the unstable dialdehyde and a further breakdown product, likely to be acrolein, may be formed. In the enzymatic reaction catalyzed by BSAO only H_2O_2 and aldehydes resulted to be responsible for cell killing. It was previously observed that BSAO and exogenous Spm caused cytotoxicity in Chinese hamster ovary (CHO) cells (Averill-Bates et al., 1993). Cytotoxicity was dependent on both, the concentration of Spm and the incubation time. H_2O_2 was considered responsible for cytotoxicity by some authors (Parchment et al., 1990), while other ones rather ascribed cytotoxicity to aldehydes (Morgan, 1987). Our results suggested that H_2O_2 is only in part responsible for cell killing since catalase only affords partial protection (Calcabrini et al., 2002). The finding also suggested that oxidation products of polyamines, rather than the polyamines themselves (Ben-Hur and Riklis, 1978), are responsible for cytotoxicity in mammalian cells (Averill-Bates et al., 1993). Moreover, the essential role in cytotoxicity of each of the enzymatic oxidation products of polyamines (H_2O_2 and aldehydes) under both normal and hyperthermic conditions was subsequently investigated (Agostinelli et al., 2006c). Cytotoxicity resulted to be accelerated at 42 °C relative to 37 °C.

Very recently results have demonstrated that the oxidation products of polyamines induce necrosis or apoptosis, inhibition of cell proliferation and inhibition of DNA and protein synthesis in several cell types (Calcabrini et al., 2002; Agostinelli et al., 2006c). The in vivo translation of these preclinical observations required, however, further investigations since BSAO has a low plasma half-life and some issues on tumour target delivery have to be solved.

In order to overcome these limits it has been evaluated, using a mouse melanoma model, whether BSAO, when injected directly into the tumour, is able to induce tumoricidal activity by converting polyamines, that are present at elevated levels in tumour tissues, to toxic products in situ (Averill-Bates et al., 2005). Moreover, several findings suggest that immobilization of enzymes, like asparaginases, into polymeric matrices such as poly ethylene glycol (PEG) increases their structural stability and functional activities in vitro and in vivo. It was reported that the immobilization of BSAO into a biocompatible matrix made of serum albumin (BSA) and PEG, showed a high operational stability relative to its native form (Demers et al., 2001). The investigation evaluates the anti-tumour activities, in vivo, of both native and PEG-BSAO in mouse melanoma and also determines the mechanism of tumour cell death. C57BL mice received a subcutaneous injection of B16 melanoma cells to induce formation of tumours, prior to anti-tumour treatments with BSAO. HPLC analysis of serum polyamines indicated that the substrates of BSAO were present in sufficient concentrations to allow the kinetic reaction to occur. Anti-tumour treatments consisted of a single injection of enzyme. When immobilized BSAO was injected into the tumour, there was a marked decrease of the tumour growth. This decrement was higher if compared with that observed when the native BSAO was administered. The time course for kinetics of apoptosis occurrence and necrosis in tumours treated with either form of BSAO showed a high level of apoptosis when immobilized BSAO was used, compared to the native enzyme. PEG-BSAO can act by allowing the slow release of cytotoxic products, which induces tumour cell death by apoptosis rather than necrosis (Averill-Bates et al., 2005).

The formation of cytotoxic products from Spm by an enzyme-catalyzed reaction is a recent approach in cancer therapy to overcome multidrug resistance (MDR) of human cancer cells. The involvement of these products in causing cytotoxicity was investigated in both drug-sensitive (LoVo WT) and drug resistant (LoVo DX) colon adenocarcinoma cells (Calcabrini et al., 2002). Transmission electron microscopic observations showed more pronounced mitochondrial modifications in drug-resistant than in drug-sensitive cells. Mitochondrial functionality studies revealed basal hyperpolarization of the mitochondrial membrane in LoVo DX cells. After treatment with BSAO and Spm higher mitochondrial membrane depolar-

ization was found in LoVo DX cells than in LoVo WT cells. Moreover, a higher basal ROS production in LoVo DX cells than in drug-sensitive cells was detected. ROS probably alter mitochondrial functionality affecting the sensitivity of cells to the cytotoxic enzymatic oxidation products of Spm (Calcabrini et al., 2002; Arancia et al., 2004).

It was also demonstrated by Maccarrone et colleagues that spermine-induced cytochrome c release from mitochondria was accompanied by dissipation of membrane potential $\Delta\Psi$ with concomitant disruption and loss of mitochondria membrane integrity. The addition of exogenous amine oxidase (AO) potentiated the effect of Spm and other polyamines on mitochondria to trigger off cytochrome c release. In fact, the effects of Spm were prevented by pargiline an inhibitor of AO (Maccarrone et al., 2001).

These findings suggest that toxic oxidation products, generated from Spm and BSAO, might be promising anticancer agents in the development of new therapeutic treatments, mainly against drug-resistant tumour cells.

New strategies based on the interference with polyamine metabolism

The involvement of polyamines in both protein synthesis machinery and intracellular production of oxidative products has pushed the design of new anti-cancer strategies based on the interference with polyamine metabolism. The disruption of the metabolic pathways downstream polyamine biosynthesis could lead to more advantageous results than the block of polyamine synthesis itself. In fact, the latter can induce unacceptable in vivo toxicity since the maintenance of sufficiently high intracellular polyamine levels is required for the protection of normal eukaryotic cells from the onset of spontaneous or injury-induced apoptosis. This consideration has severely limited the development in clinical settings of drugs that inhibit polyamine biosynthesis. The specific interference with selected processes dependent from polyamine-dependent reactions and involved in the regulation of tumour growth could be more suitable for clinical translation.

The block of hypusine formation in eIF5A as a new way for anti-cancer intervention

Evidence has been accumulated on the role of eIF5A in the regulation of apoptosis and proliferation. Therefore, the specific targeting of eIF5A function through the inhibition of hypusine formation could be a novel strategy in order to improve anti-cancer activity of anti-tumour agents.

We have reported that IFNα, a common cytokine widely used in the therapy of human cancers (Kirkwood et al., 1997), induces growth inhibition and increased expression of the epidermal growth factor receptor (EGF-R). At the same time, EGF antagonizes IFNα-induced apoptosis through an EGF-ras-Erk-dependent pathway (Caraglia et al., 2003b). In the same experimental conditions, the treatment of human epidermoid cancer cells reduces the activity of eIF5A (evaluated through the determination of hypusine levels) while the addition of EGF to IFNα-treated cells restores hypusine levels (Caraglia et al., 1997). This latter effect could reduce the activity of eIF5A with a consequent decrease of the efficiency of protein synthesis process and could be at least in part responsible for the apoptosis caused by IFNα. On the basis of these observations, we have used in combination with IFNα the recombinant fusion protein TP-40, formed by transforming growth factor α, which delivers the protein to EGF-R, and the toxin of *Pseudomonas Aeruginosa* which affects protein synthesis through the inhibition of the elongation factor 2 (EF-2). We have indeed found a synergism between TP-40 and IFNα on the cytotoxicity of KB cells and an increased uptake of TP-40 by tumour cells treated by IFNα (Caraglia et al., 1997). This strategy could be useful in the treatment of human epidermoid cancers taking advantage of both an improved tumour tissue targeting (obtained through the increased expression of EGF-R as tumour associated antigen) and the potentiation of protein synthesis inhibition (through a multi-step inhibition). Moreover, EGF antagonism on apoptosis induced by the cytokine was paralleled by a restoration of hypusine synthesis caused by the cytokine and an increase of extracellular signal regulated kinase (ERK) activity in the same cancer cells. In the same experimental conditions, we have also found that PD098059, a specific inhibitor of mitogen extracellular signal regulated kinase (MEK-1) and thus of ERK, reduces hypusine synthesis and enhances the decrease of intracellular hypusine content caused by the cytokine (Caraglia et al., 2003a). Moreover, PD098059 is also able to antagonize the recovery of hypusine synthesis induced by EGF (Caraglia et al., 2003a). The reduction of hypusine synthesis could be even higher if tumour cells treated with IFNα did not show an anti-apoptotic response based on the hyper activation of the MEK-ERK pathway. Therefore, the addition of PD098059 to IFNα-pre-treated cells overcomes this survival pathway inducing a potentiation of both hypusine level reduction and apoptosis. On the other hand, the addition of EGF to IFNα-treated cells over stimulated this survival pathway inducing a recovery of

both hypusine levels and apoptosis (Caraglia et al., 2003a). Therefore, the selective inhibition of ras with gene transfer therapeutic strategies based on the delivery of dominant negative forms of ras such as RASN17 or with agents that block ras farnesylation such as the farnesyltransferase inhibitors (FTI) could be also considered in order to enhance the antiproliferative action of IFNα. MEK-1 and consequently the activation of ERK-1/2 could be also evaluated as additional target through the use of selective inhibitors such as PD098059. Finally, on the basis of the previous findings, we can also hypothesize that the selective interference on eIF5A activity could be an additional target in order to potentiate the antitumor efficacy of IFNα (Caraglia et al., 2005) (Fig. 1). In fact, we have found that the specific deoxyhypusine synthase inhibitor GC7 synergizes with IFNα in inducing cell growth inhibition and apoptosis suggesting a critical role for eIF5A in the modulation of cell proliferation induced by IFNα in human epidermoid cancer cells (Caraglia et al., 2003a). All these data support the potential use of the

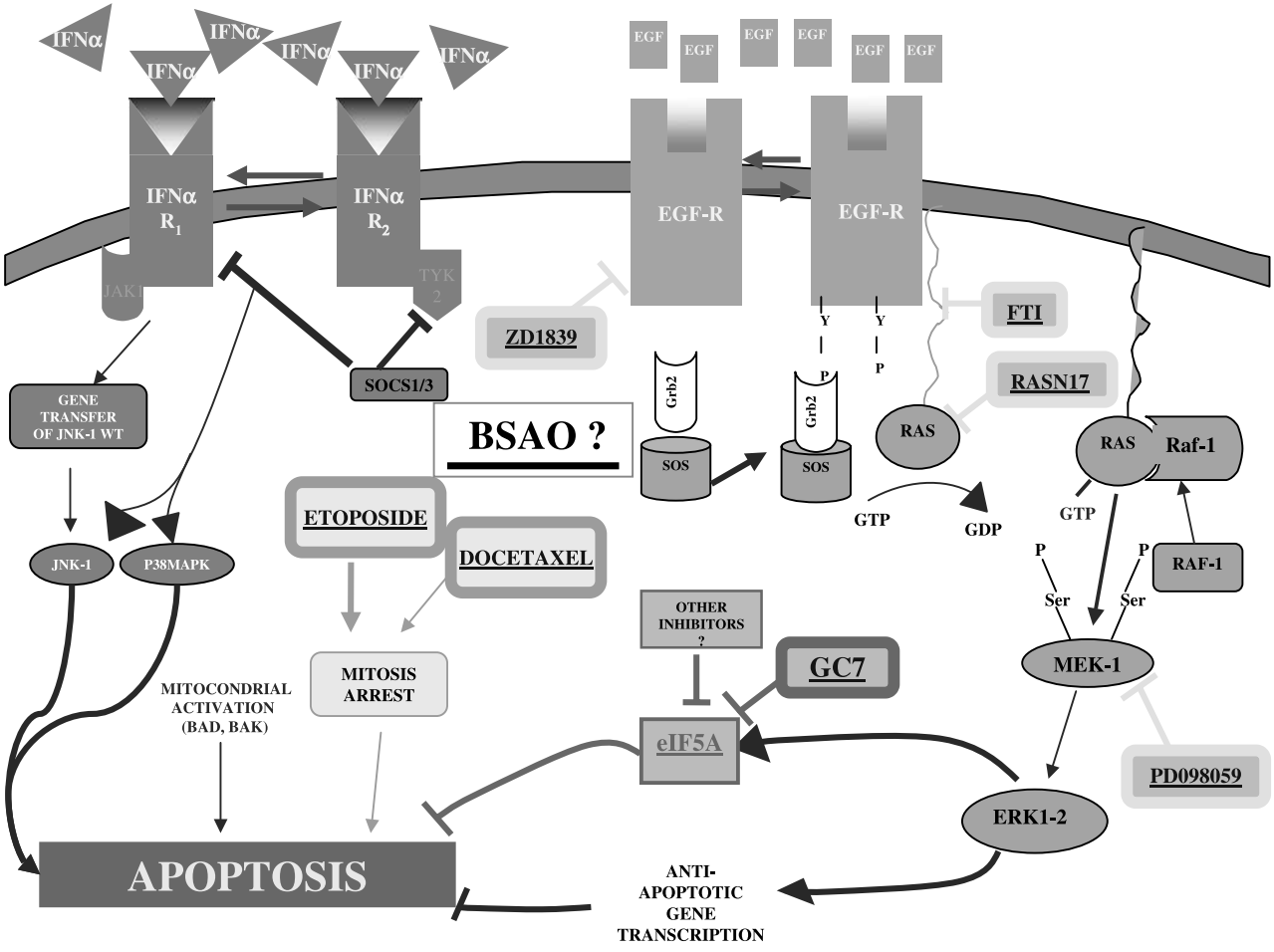

Fig. 1. *Left*: Induction of apoptosis induced by IFNα likely through the activation of caspase cascade mediated by stress-dependent kinase JNK-1 and/or p38MAPK stimulation and through the involvement of mitochondrial proteins as Bad and Bak. *Right*: The EGF-dependent proliferation pathway that triggers a ras-dependent ERK-1/2 activation that inhibits IFNα-induced apoptosis probably counteracting on caspase cascade activation. Moreover, ERK-1/2 can stimulates the activity of eIF5A that displays anti-apoptotic activities. The inhibition of this pathway through the use of the EGF-R-associated tyrosine kinase inhibitor ZD1839 or the dominant negative ras RASN17 or FTIs or the MEK-1 inhibitor PD098059 induces the release of this anti-apoptotic pathway with the subsequent potentiation of the apoptosis. Also the selective inhibition of eIF5A with the hypusine synthesis inhibitor GC7 or with other specific inhibitors to be found could enhance the apoptotic mechanism. *Middle*: The BSAO has a role not still known within these pathways, but using the combinations of several anti-cancer drugs, as etoposide or docetaxel that act preventing mitosis, with BSAO/endogenous polyamines it could be possible to improve the activity of both H_2O_2 and aldehydes and to enhance cell death. → Stimulating activity, ⊣ Inhibiting activity. Squares show the possibilities of therapeutic interventions in order to increase the antiproliferative activity of IFNα. *EGF* epidermal growth factor; *EGF-R* EGF receptor; *FTI* farnesyltransferase inhibitor; *RASN17* dominant negative ras plasmid; *TCF* ternary complex factor; *Erk* extracellular signal regulated kinase; *Mek* mitogen extracellular signal regulated kinase; *eIF5A* eukaryotic initiation factor of protein synthesis 5A; *GC7* 1-guanyl-1,7-diaminoheptane; *JNK-1* Jun kinase-1; *p38 MAPK* p38 mitogen activated protein kinase

polyamine-dependent modifications of eIF5A in experimental models of therapy of human epidermoid cancer cells (Caraglia et al., 2004).

The formation of oxidation products from polyamines potentiates anti-cancer activity of chemotherapy drugs

From a therapeutic point of view the in situ improvement of the activity of polyamine metabolites deserves consideration. Using the combinations of several anticancer drugs or heat with BSAO/endogenous polyamines could induce activity improvement of both H_2O_2 and aldehyde(s).

Cell survival experiments performed on cells treated with BSAO/spermine in combination with hyperthermia showed, as reported above, that cytotoxicity was considerably enhanced at 42 °C and was higher in MDR adenocarcinoma cells than in their drug-sensitive counterparts (Agostinelli et al., 2004, 2006a). Moreover, findings were already published suggesting a new strategy to overcome MDR of human cancer cells by using BSAO/Spermine in association with N^1,N^4-bis(2,3-butadienyl)-1,4-butanediamine (MDL 72527) (Agostinelli et al., 2006b). Experiments performed on human adenocarcinoma cancer cells pre-treated with 300 μM MDL 72527 for 24 h and then with BSAO/spermine at 37 °C, clearly showed that cytotoxicity was higher in MDR cells than in their sensitive counterparts. Cytotoxicity was considerably enhanced using the association BSAO/spermine and MDL 72527, if compared to the cytotoxic effect induced by the sole enzymatic system.

Moreover, current trends in the treatment of human tumours are for drug combinations that result in improved responses as well as the ability to use less toxic concentrations of the drugs. Induction of apoptosis in cancer cells induced by chemotherapy agents such as etoposide (VP16) and docetaxel (DTX) was paralelled by an increase in the level of intracellular peroxy radicals and lipid peroxidation products (Limoli et al., 1998) two independent end points that are typically associated with oxidative stress. Similar findings were observed in several sub-clones showing persistent apoptosis. On the basis of these data, the combined treatment between the BSAO/Spermine enzymatic system and different anti-cancer drugs as VP16 or DTX is under investigation in order to induce strong cytotoxic effects using lower concentrations of the cytotoxic drugs (Fig. 1).

DTX is an antineoplastic agent belonging to the taxoid family that promotes the assembly of tubulin into stable microtubules while simultaneously inhibiting their disassembly in the absence of GTP (Lyseng-Williamson and Fenton, 2005). This leads to a significant decrease in free tubulin, needed for microtubule formation and results in inhibition of mitotic cell division between metaphase and anaphase, preventing further cancer cell progeny (Lyseng-Williamson and Fenton, 2005). Apoptosis is also encouraged by the blocking of apoptosis-blocking bcl-2 oncoprotein (Lyseng-Williamson and Fenton, 2005). Both in vitro and in vivo analysis show the anti-neoplastic activity of DTX to be effective against a wide range of known cancer cells, cooperate with other anti-neoplastic agents activity, and have greater cytotoxicity than paclitaxel, possibly due to its more rapid intracellular uptake (Lyseng-Williamson and Fenton, 2005). The main mode of therapeutic action of DTX is the suppression of microtubule dynamic assembly and disassembly, rather than microtubule bundling leading to apoptosis, or the blocking of bcl-2 (Lyseng-Williamson and Fenton, 2005).

VePesid® (VP16) (also commonly known as VP-16) is a semi-synthetic derivative of podophyllotoxin that interferes with the synthesis of DNA and has a secondary effect on arresting cells in resting (G2) phase. VP16 rapidly induces production of intracellular ROS in HS-27A stromal cells and is required for conversion of pro-MMP-2 to its active form (Wang et al., 2005).

Our aim is to evaluate if the cytotoxicity of anti-cancer agents VP16 or DTX could be potentiated by treatment of tumour cells with BSAO/SpM enzymatic system in human head and neck KB and breast MCF-7 cancer cells. Subsequently, we will investigate the basis of the growth inhibition induced by drug combination and the levels of intracellular reactive oxygen species (ROS) to demonstrate that the use of drugs in combination allows the decrease of the anti-tumour active dose of the single molecules with no additive cytotoxic effects.

Conclusions and perspectives

On the basis of the previous findings, we can hypothesize that the selective interference on eIF5A activity could be an additional target in order to potentiate the anti-tumour efficacy of IFNα. In fact, we have found that hypusine synthesis inhibitor, and thus eIF5A inactivator, GC7 synergizes with the cytokine in the induction of cell growth inhibition and apoptosis (Caraglia et al., 2003a).

It has been recently performed a computer-based prediction of the three dimensional structure of eIF5A in order to define the structure of the hypusine-containing site (Facchiano et al., 2001). We are now planning a phar-

macological screening of drugs with potential eIF5A-inhibiting properties.

On the basis of these findings, we hypothesize that the selective interference on eIF5A activity either through the blocking of hypusine synthesis (mediated by agents similar to GC7) or the selective binding with the hypusine-containing site could represent a new scenario of intervention in anticancer therapy based on IFNα administration (Fig. 1).

Another unexplored possibility, in clinic therapy, is that cytotoxic enzymatic oxidation products of Spm in combination with different drugs might be promising anticancer strategies. Furthermore, the encapsulation of BSAO in pegylated liposome and subsequent serum delivery could allow the targeting of tumour tissues. On the basis of our previous findings, different therapeutic strategies are under preclinical investigation in order to increase the anticancer activity of BSAO/Spm enzymatic system. In conclusion, the understanding of the molecular mechanisms regulating apoptosis and cell growth inhibition mediated by polyamine metabolites could be useful in the design of new therapeutic strategies based on the use of BSAO/Spm enzymatic system.

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