Amino Acids (2012) 42:441–450
DOI 10.1007/s00726-011-1014-z

MINIREVIEW ARTICLE

Spermine oxidase: ten years after

Manuela Cervelli · Roberto Amendola · Fabio Polticelli · Paolo Mariottini

Received: 4 March 2011 / Accepted: 25 May 2011 / Published online: 2 August 2011

Abstract Spermine oxidase (SMO) was discovered much more recently than other enzymes involved in polyamine metabolism; this review summarizes 10 years of researches on this enzyme. Spermine oxidase (SMO) is a FAD-dependent enzyme that specifically oxidizes spermine (Spm) and plays a dominant role in the highly regulated mammalian polyamines catabolism. SMO participates in drug response, apoptosis, response to stressful stimuli and etiology of several pathological conditions, including cancer. SMO is a highly inducible enzyme, its deregulation can alter polyamine homeostasis, and dysregulation of polyamine catabolism is often associated with several disease states. The oxidative products of SMO activity are spermidine, and the reactive oxygen species H_2O_2 and the aldehyde 3-aminopropanal each with the potential to produce cellular damages and pathologies. The SMO substrate Spm is a tetramine that plays mandatory roles in several cell functions, such as DNA synthesis, cellular proliferation, modulation of ion channels function, cellular signaling, nitric oxide synthesis and inhibition of immune responses. The goal of this review is to cover the main biochemical, cellular and physiological processes in which SMO is involved.

Keywords Spermine oxidase · Spermine · Gene expression · Enzyme activity · Inhibitors · Differentiation · Cancer · Brain

Abbreviations

AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid
AP	Aminopropanal
APAO	N^1-acetylspermine oxidase
BC	Breat cancer
BENSpm	Bis(ethyl)norspermine
CPENSpm	N^1-ethyl-N^{11}-(cyclopropyl)-methyl-4,8-diazaundecane
ETBF	Enterotoxigenic *Bacteroides fragilis*
FAD	Flavin-adenine-dinucleotide
FMS1	Fenpropimorph resistance multicopy suppressor 1
Kir	Inward rectifier K^+ channels
H_2O_2	Hydrogen peroxide
MDL 72527	N^1,N^4-bis(2,3-butadienyl)-1,4-butanediamine
NB	Neuroblastoma
NMDA	N-methyl-D-aspartate
ODC	Ornithine decarboxylase
PA	Polyamines
PC-Acro	Protein-conjugated acrolein
ROS	Reactive oxygen species
SBI	Silent brain infarction
Spd	Spermidine
SMO	Spermine oxidase

M. Cervelli · F. Polticelli · P. Mariottini (✉)
Department of Biology, University Roma Tre,
00146 Rome, Italy
e-mail: mariotpa@uniroma3.it

M. Cervelli
e-mail: cervelli@uniroma3.it

F. Polticelli
e-mail: polticel@uniroma3.it

R. Amendola
ENEA, CR Casaccia, BAS.BIOTEC MED, 00123 Rome, Italy
e-mail: roberto.amendola@enea.it

F. Polticelli
National Institute for Nuclear Physics, Roma Tre Section,
00146 Rome, Italy

SMS	Spermine synthase
Spm	Spermine
SSAT	Spermidine/spermine N^1-acetyltransferase
TBI	Traumatic brain injury
TNF-α	Tumor-necrosis factor-α
ZmPAO	Maize polyamine oxidase

Introduction

Mammalian spermine oxidase (SMO, EC number 1.5.3.16) is a flavoprotein, which specifically oxidizes spermine (Spm), with the production of spermidine (Spd), hydrogen peroxide (H_2O_2) and the aldehyde 3-aminopropanal (3-AP) (Fig. 1). The human SMO cDNA was first cloned and characterized by Wang et al. 2001 and initially named PAOh1 (Wang et al. 2001). After the substrate specificity for Spm was confirmed, it was subsequently renamed SMO (Vujcic et al. 2002; Cervelli et al. 2003; Wang et al. 2005). This enzyme plays a central function in the highly regulated polyamines (PA) catabolism that involves two more proteins, the inducible spermidine/spermine N^1-acetyltransferase (SSAT) and the constitutively expressed N^1-acetylpolyamine oxidase (APAO) (Casero and Pegg 1993; Wallace et al. 2003). In the last decade, SMO, APAO and SSAT catabolic enzymes have been extensively characterized and it is well documented that these enzymes play an essential role in maintaining vertebrate PA homeostasis, which is mandatory for cellular life (Wallace et al. 2003; Agostinelli et al. 2004; Seiler 2004a; Casero and Marton 2007; Amendola et al. 2009; Casero and Pegg 2010). SMO is a highly inducible enzyme by a variety of stressful stimuli, including several antitumor PA analogs (Wang et al. 2001; Vujcic et al. 2002; Devereux et al. 2003; Casero and Marton 2007; Casero and Pegg 2010). It is well ascertained that this enzyme participates in drug response, apoptosis, etiology of several pathological conditions, including cancer, since its dysregulation can alter PA homeostasis affecting PA catabolism, which has been observed to be often associated with several disease states (Amendola et al. 2009; Agostinelli et al. 2010; Casero and Pegg 2010). In line with this, Spm degradation seems to be of vital importance, since mice die after prolonged inhibition of PA catabolism (Seiler 2004b). Both human and mouse cDNAs have been expressed in *Escherichia coli* cells and the corresponding enzymes have been biochemically characterized (Cervelli et al. 2004; Bellelli et al. 2004; Bianchi et al. 2005, 2006; Tavladoraki et al. 2010; Adachi et al. 2010a). Further, a considerable effort has been made to better understand the structure–function relationships of the mammalian SMO enzyme-substrate complex, probed through a combination of molecular modeling, site-directed mutagenesis and biochemical characterization studies (Tavladoraki et al. 2010). Some inhibitors of SMO have been used to dissect its precise role in PA metabolism and related pathologies (Casero and Marton 2007; Amendola et al. 2009; Casero and Pegg 2010). However, none of the available SMO inhibitors displays the desired characteristics of selective affinity and specificity. In addition, the development of novel, more specific, SMO inhibitors has been prevented by the lack of a crystal structure for the SMO protein, as repeated efforts to obtain structural details of this enzyme at the atomic level have all failed (Tavladoraki et al. 2010). A good deal of data has been also gathered on the physiological role of SMO, which is considered the primary source of cytotoxic H_2O_2 when PA catabolism is induced by PA analogs or by inflammation or infectious agents, such as *Helicobacter pylori* (Chaturvedi et al. 2004; Pledgie et al. 2005). SMO can also play a relevant function in chronic inflammation since the concomitant increase in reactive oxygen species and oxidative damage are estimated to contribute to the etiology of at least 20% of human cancer cases (Goodwin et al. 2008a). It is consequently highly desirable to have specific PA analogs that act as inhibitors of the SMO enzyme in order to both dissect its precise role in PA metabolism and, more importantly, in cases in which SMO induction is related to carcinogenesis, to lower SMO enzymatic activity as a therapeutic treatment. The SMO substrate Spm is the largest molecule involved in the PA metabolism and it contributes to maintain the cellular physiological status, since it can act as a regulator of DNA synthesis, cellular proliferation, as a second messenger in cellular signaling, as a modulator of the synthesis of nitric oxide and as an inhibitor in immune responses. In line with the notion that SMO enzyme is highly expressed in the brain (Cervelli et al. 2004), it is well documented that Spm plays also an important role in brain functions, modulating the response of several glutamate receptors, and Kir and Na^+ channels (Williams 1997; Masuko et al. 2003; Han

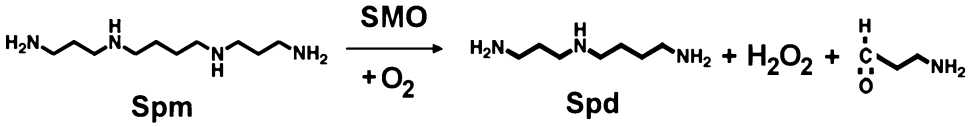

Fig. 1 Enzymatic reaction catalyzed by SMO. The physiological substrate Spm is oxidized to Spd with production of 3-AP and hydrogen peroxide

et al. 2008). Accumulating evidence suggests that the PA system also plays a role in the etiology and pathology of mental disorders. Alterations in the expression and activity of PA metabolic enzymes, as well as changes in the levels of the individual polyamines, have been observed in multiple conditions, including schizophrenia, mood disorders, anxiety and suicidal behavior. The aim of this review is to highlight the main biochemical and cellular functions of SMO.

SMO gene structure, expression and regulation

The single copy mammalian *SMO* gene (GenBank name SMOX) encompasses seven exons, and it is located on chromosome 2 in human and has adopted the evolutionary mechanism of alternative splicing to increase its functional variation. In fact, *SMO* gene codes for multiple splice variants (Fig. 2) and two catalytically active isoforms have been characterized: SMO1 and SMO5 in human, SMOα and SMOμ in mouse (Murray-Stewart et al. 2002, 2008; Cervelli et al. 2004). Interestingly, these splice variants show different subcellular localization: SMO1, SMO5 and SMOμ are found both in the cytoplasm and in the nucleus, while SMOα was proven to be localized only in the cytoplasm (Bianchi et al. 2005; Murray-Stewart et al. 2008). SMO expression appears to be regulated predominantly at the transcriptional level and by messenger RNA stabilization, and it has been shown that tumor-necrosis factor-α (TNF-α) can induce H_2O_2 production via *SMO* gene up-regulation (Wang et al. 2005; Babbar and Casero 2006). In general, SMO expression is induced during cellular stress, like inflammation and DNA damage, but it has been shown that SMO transcript accumulation increases also during mouse myoblast C2C12 cell differentiation (Cervelli et al. 2009). On the contrary, in proliferating cells *SMO* gene expression appears to be low, as in breast cancer tissues where it may contribute to tumor growth through a decrease in the local H_2O_2 concentration (Cervelli et al. 2010). These issues will be discussed in more detail below.

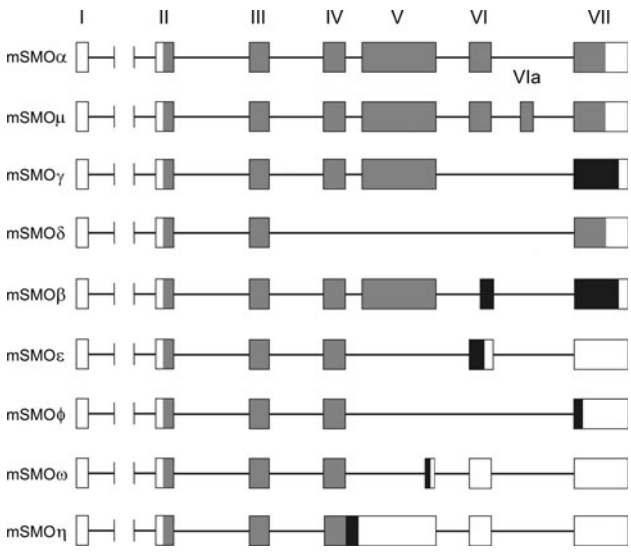

Fig. 2 Exon structures of murine SMO splice variants. Schematic representation not in scale. *Open and dark gray boxes* represent noncoding and coding sequences, respectively. *Black boxes* represent different ORFs

SMO biochemical features

Recombinant forms of human and mouse SMO (SMO1 and SMOα, respectively) have been first expressed and characterized independently by Wang et al. (2003) and by Cervelli et al. (2003), respectively, who demonstrated that SMOs are able to oxidize Spm to Spd and 3-AP. On the contrary, both enzymes fail to oxidize Spd and N^1-acetyl-polyamines, thus demonstrating a unique substrate specificity with respect to other animal polyamine oxidases such as the yeast enzyme Fenpropimorph resistance multicopy suppressor 1 (FMS1) and the mammalian APAO (Henderson Pozzi et al. 2009; Adachi et al. 2010b). In particular, SMOα has been the subject of intense studies in the last few years. SMOα displays an optimal pH value of 8.0 and *Km* and k_{cat} values of 90 μM and 4.5 s^{-1}, respectively, at pH 8.0 (Cervelli et al. 2003). From a structural viewpoint, attempts from independent laboratories to obtain protein crystals for X-ray diffraction studies failed, likely due to the presence of highly flexible loop regions (Tavladoraki et al. 2010). However, SMOα displays a significant sequence similarity with the structurally characterized polyamine oxidase from maize (ZmPAO, 41% sequence similarity), a feature that allowed to build a molecular model of SMOα three-dimensional structure (Fig. 3) (Cervelli et al. 2003; Tavladoraki et al. 2010). The modeled SMOα active site resembles that of FMS1 for the presence of His82 and Tyr482, which are conserved in FMS1 as His67 and Tyr450 (Fig. 4) (Huang et al. 2005), and this allowed also to construct a model of the SMOα-Spm complex that was successfully validated through site-directed mutagenesis studies (Tavladoraki et al. 2010). According to this model, the substrate is bound in the correct position to undergo oxidation through a series of electrostatic interactions involving the substrate amino groups and the protein residues His82, Gln200, Glu224, Tyr482 and Ser527 (Fig. 4). SMOα-Spm structural model predicts that Spm is bound in an U-shape conformation around His82 and indeed mutation of this residue almost abolishes enzyme activity (Tavladoraki et al. 2010). Finally, SMOα, as all the PAO-like enzymes, displays the conservation of Lys367, orthologous to the Lys300 residue

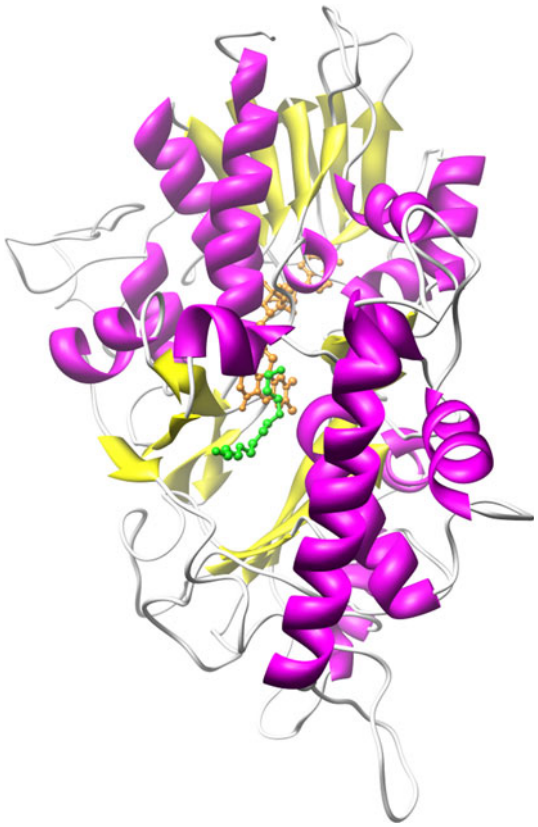

Fig. 3 Schematic representation of the three-dimensional model of SMOα-Spm complex. The enzyme displays the typical fold of flavin-dependent amine oxidases characterized by mixed *α*/*β* FAD-binding and substrate-binding domains. FAD is colored in *orange* and Spm in *green*

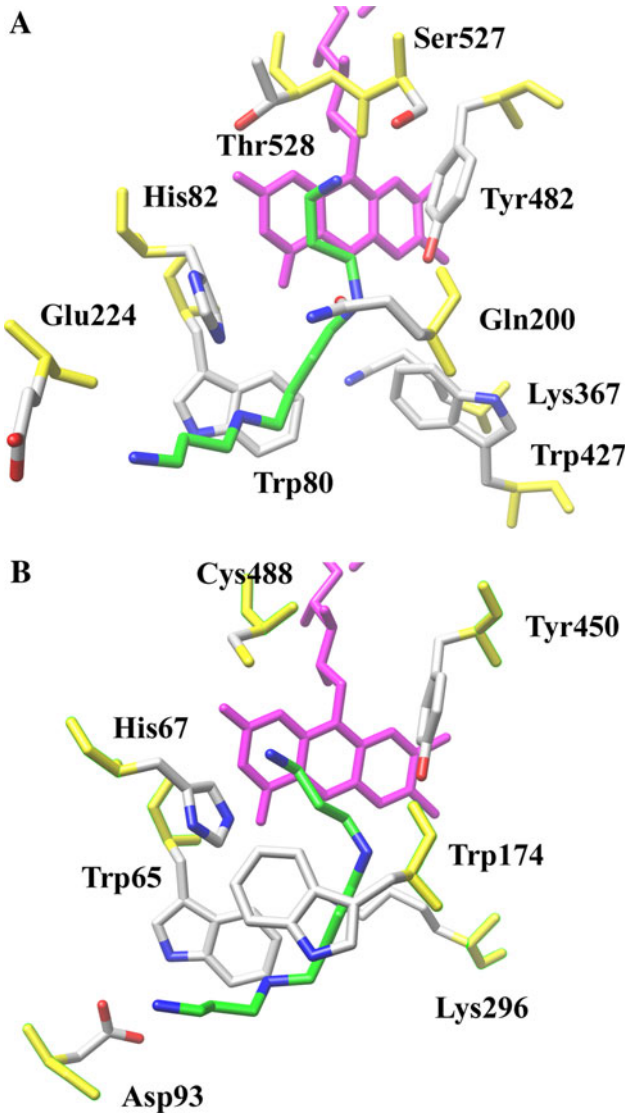

Fig. 4 Schematic representation of the active site structure of SMOα-Spm (**a**) and yeast FMS1-Spm (**b**) complexes. For clarity, the FAD moiety is represented in *purple*, the Spm carbon atoms in *green* and the backbone atoms of the active site residues in *yellow*

of ZmPAO, which in the latter protein forms the Lys-H_2O-FAD structural motif essential for catalysis (Polticelli et al. 2005). Also in SMOα, Lys367 plays a relevant role in the catalytic mechanism as mutation of this residue to Met causes a substantial decrease in k_{cat} and a shift of the pH profile of the activity (Tavladoraki et al. 2010). In this regard, pK_as calculations support the hypothesis that substrate deprotonation, facilitated by electrostatic interactions with Lys367, is responsible for the observed pH dependence of the activity. In this regard, results reported in a recent, very detailed mechanistic study of human SMO1 by Adachi et al. (2010a), indicate that the triprotonated form of Spm (one of the secondary amino groups being deprotonated) is the active substrate. The authors propose that this feature is at the basis of the preference of SMOs for Spm over N^1-acetyl-Spm, in which the terminal acetylated amine group is unprotonated (Adachi et al. 2010a). This may well be the case and indeed molecular modeling studies highlight structural differences between SMOs and APAOs, which provide a complementary explanation for SMOs substrate selectivity (Tavladoraki et al. 2010). In particular, according to this study, SMO active site is characterized by a negatively charged specificity pocket which allows binding of Spm, possessing a protonated primary amino group, but negatively selects N^1-acetyl-Spm in which the corresponding group is neutral and possesses a hydrophobic methyl group.

SMO inhibitors

As already mentioned in the "Introduction" section, PA levels affect cell growth, differentiation and apoptosis, and defects in the metabolism of PA have been linked to cancer (see Casero and Marton 2007 and Amendola et al. 2009 for recent reviews). Thus, it is clear the need for specific

Table 1 K_i values for selected murine SMO and APAO inhibitors

Inhibitor	APAO K_i (M)	SMO K_i (M)
1,8-Diaminooctane	$>10^{-2}$	$>10^{-2}$
1,12-Diaminododecane	8.0×10^{-6}	$>10^{-3}$
N-prenylagmatine	8.0×10^{-7}	4.6×10^{-5}
Guazatine	4.5×10^{-7}	4.0×10^{-7}
MDL72527	2.1×10^{-5}	6.3×10^{-5}
BENSpm	N.A.	3.8×10^{-4}
CPENSpm	N.A.	8.5×10^{-5}

N.A. not available

inhibitors of SMO and APAO which would allow to analyze the precise role of each of these enzymes in the metabolism of PA. Several inhibitors, belonging to different structural classes, have been tested as modulators of SMOα activity. In particular, the inhibitory properties of linear primary diamines lacking secondary amino groups, agmatine derivatives, di-guanidino compounds and N^1, N^4-bis(2,3-butadienyl)-1,4 butanediamine (MDL 72527), a Spm analog which lacks terminal amino groups, have been reported in a comparative study on ZmPAO, SMOα and mouse APAO (Table 1) (Bianchi et al. 2006 and references therein). Linear diamines, surprisingly including the Spm isosteric compound 1,12-diaminododecane, do not inhibit SMOα, likely due to the absence of secondary amino groups that, according to modeling studies, are required for binding to occur in the vicinity of His82 (Fig. 4) (Tavladoraki et al. 2010). It is worthwhile to mention that 1,12-diaminododecane inhibits competitively mouse APAO ($K_i = 8.0 \times 10^{-6}$ M), and thus, it represents a good candidate for specific inhibition of this enzyme (Bianchi et al. 2006). However, of all the inhibitors tested in this study, none possesses the desired affinity and specificity for SMOα to act as a selective inhibitor of this enzyme, as both di-guanidino compounds and agmatine derivatives, as well as MDL 72527, inhibit both murine SMOα and APAO with similar K_i values (Bianchi et al. 2006). Two Spm analogs, namely bis(ethyl)norspermine (BENSpm) and N^1-ethyl-N^{11}-(cyclopropyl)-methyl-4,8-diazaundecane (CPENSpm), have been tested as SMOα inhibitors. However, these inhibitors display poor affinity for the enzyme, and their K_i values being in the 10^{-4} to 10^{-5} M range (Table 1) (Cervelli et al. 2010,). The search for specific SMO inhibitors is therefore still an important open issue. Recently, a set of N-alkylated polyamine analogs used for the treatment of proliferative disorders or parasitic diseases have been tested as SMO substrates (Häkkinen et al. 2010). SMO is revealed to be capable of metabolizing most of them, suggesting that it may have a role in drug-mediated cytotoxic response.

SMO in differentiation and cancer

The PA content is strictly related to cell growth and differentiation with a consistent number of evidences that relate PA metabolism dysfunction with cancer (Thomas and Thomas 2003; Gerner and Meyskens 2004; Agostinelli et al. 2010). Cancer cells of different histological types often show altered pathways of cell growth and differentiation. Knowledge of the mechanisms involved in maintaining the appropriate differentiation pathways in normal cells and how such mechanisms are subverted in malignant cells is increasingly important, both for prognosis and for therapy (Barakat et al. 2010). Cellular differentiation is accomplished by the loss of proliferative activity and the acquisition of specialized functions, which are often manifested in the context of a multicellular tissue organization. Therefore, it is important to understand the mechanisms underlying the inability of tumor cells to differentiate and, conversely, how differentiation-inducing agents can be used to restore a more normal phenotype in these cells (Lotan et al. 1990). Potentially, each PA's biosynthetic and catabolic step is a target for such a kind of therapies, as well as PA transport across cell membrane, to minimize the homoeostatic effects of the PA metabolism (Casero and Marton 2007; Amendola et al. 2009). Enhanced activity of SMO enzyme has been reported to correlate with differentiation in the skeletal muscle C2C12 cell line model, with the decrease in Spm and augmented level of reactive oxygen species (ROS). Since ROS are continually generated in muscle, it is believed that these molecules have a well-established role as physiological modulators of skeletal muscle functions (Cervelli et al. 2008). Similar evidences have been collected from a neuroblastoma (NB) cell line. Neuroblastoma, the most common pediatric solid tumor, often retains the differentiation and developmental potential characteristic of primitive neural crest cells, making its clinical outcome extremely variable (Amendola et al. 1997). In the mouse NB N18TG2 cell line, SMO and SSAT enzymatic activities correlate with enhanced differentiation (Amendola R., unpublished results). These preliminary results are in line with data coming from clinical evaluation of ornithine decarboxylase (ODC) activity. Diminished levels of ODC activity, independent from the transcription factor N-Myc, but related to the ODC inhibitory effect of antizyme-2, represent a marker of positive diagnostic outcome (Geerts et al. 2010). ODC activity is inversely related to differentiation and, generally, to both SSAT and SMO activities (Pledgie et al. 2005). Nowadays, the most important link established between cancer and SMO activity is inflammation. Inflammation has been implicated in the development of many human epithelial cancers, including those of lung, prostate, stomach and colon, and an increasing number of

experimental evidences place in strong relation inflammation and DNA damage through the production of H_2O_2 by SMO. Both TNF-α and interleukin-6, pleiotropic and pro-inflammatory cytokines, are produced in response to inflammation and directly linked to SMO. In fact, treatment with the specific PAOs inhibitor MDL 72527 or targeting SMO by siRNA inhibit ROS overproduction and TNF-α activation in human lung epithelial cells, suggesting that a common mechanism driven by inflammation is the cause of carcinogenesis for the development and progression of epithelial lung cancers (Babbar and Casero 2006). In prostate cancer, Spm is present in high concentrations and SMO expression is an early event in the development of this tumor form, as determined by specific SMO antisera reactivity in prostate cancer tissue microarrays (Goodwin et al. 2008a). Interestingly, also the inflammation cascade following or concomitant to an infection event can lead to cancer mediated by ROS overproduced by SMO overactivity. The first, clear example derived from the observation of gut macrophages infected with *Helicobacter pylori* (Xu et al. 2004). The mechanism of macrophages apoptotic activation pathway by *Helicobacter pylori* was associated with the H_2O_2 produced by SMO and subsequent mutagenic DNA damage for carcinogenesis (Xu et al. 2004). Another cancer etiology deriving from bacterial colonization and inflammation is colon cancer. Enterotoxigenic *Bacteroides fragilis* (ETBF) colonization is associated with active inflammatory bowel disease (Prindiville et al. 2000; Basset et al. 2004) and colorectal cancer (Toprak et al. 2006). Furthermore, exposure of human colon epithelial cells to EBTF resulted in ROS-induced DNA damage through SMO overactivity (Goodwin et al. 2008b). A different scenario has been depicted in breast cancer (BC). *SMO* gene expression in BC tissues apparently plays the opposite role in apoptotic induction. In fact, SMO transcriptional level and enzymatic activity are significantly lower than in controls, and they may contribute to tumor growth through a decrease in the local H_2O_2 concentration (Cervelli et al. 2010). This result is in line with the previously obtained evidence of an APAO enzyme activity significantly lower in BC tissue (Wallace et al. 2000). A low level of APAO, associated with the increase in SSAT, was indicative of a poor prognosis (Wallace et al. 2000). The experimental and clinical evidences described above suggest that SMO can play a pivotal role in cancer development and aggressiveness. In cases of inflammation and infection-induced inflammation, SMO is involved in carcinogenesis being a source of ROS-induced DNA damage. In BC, SMO is a local death inducer, thus, representing a negative marker when expressed at low level. To this end, the interaction between Spm analogs and SMO is of therapeutic interest. The two Spm analogs, BENSpm and CPENSpm, are capable to produce cytotoxicity on some human BC cell lines by SMO induction, but they resulted to be inhibitors of SMO enzymatic activity when tested in an in vitro system (Amendola et al. 2009; Cervelli et al. 2010). The inhibition properties of these compounds could explain their failure in clinical trials. The innate capability of SMO to cause a local amount of ROS captured the interest of radio-biologists looking for an endogenous agent able to stimulate an adaptive response to radiation. The adaptive response, if triggered, can be of deleterious effect in radiotherapy, especially if associated with the enhanced level of SMO and PA metabolism described in tumors. Indeed, in a NB cell line, SMO overexpression induces a chronic sub-lethal DNA damage that causes radiosensitivity (Amendola et al. 2005; Bianchi et al. 2007). This effect is inhibited by MDL 72527 confirming the involvement of SMO in radiosensitivity.

SMO and brain

A high expression level of the SMO enzyme has been found in the brain (Cervelli et al. 2004), and the significant presence of this enzyme has important consequences on substrate regulation and products release in this organ. Independently from its function in the basal PA metabolism, the SMO substrate Spm has an important role in brain functions, since intracellular Spm is responsible for intrinsic gating and rectification of strong inward rectifier K^+ channels (Kir) by directly plugging the ion channel pore (Williams 1997; Masuko et al. 2003; Han et al. 2008). Intracellular Spm can also cause inward rectification at some subtypes of alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) and kainate Ca^{2+}-permeable receptors in the Central Nervous System, again by plugging the receptor channel pore. Extracellular Spm has multiple effects at the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors, including a stimulation that increases the intensity of NMDA receptor currents and voltage-dependent block (Williams 1997; Masuko et al. 2003; Han et al. 2008). In recent years, gene expression, genetic association and metabolic studies have implicated the PA system in psychiatric conditions, including suicidal tendencies. Suicidal behaviors are directly associated with psychiatric disorders (Fiori et al. 2010 and references therein). These behaviors, which comprise ideation, attempts and completed suicide, are among the most devastating consequences of psychiatric disorders and account for over a million deaths worldwide each year. Over the years, considerable efforts have been put toward identifying genes and pathways involved in the pathology and etiology of psychiatric disorders in order to identify new potential target genes for pharmaceutical treatments (Fiori et al. 2010). Mostly owing to the elucidation of the

molecular targets of effective psychopharmaceutical agents, much of the neurochemical work in mental disorders to date has focused on the role of the monoaminergic system. Despite the success of monoamine-related pharmacologic treatments, they are not effective in many patients, indicating that the monoaminergic system is not the only factor involved in these conditions. The significant role in mental illness of an alternative pathway, the PA system, has recently gained support. PA metabolism actually represents an important source for neurobiological factors involved in mood disorders, anxiety disorders and suicide (Fiori et al. 2010). In this regard, analysis of the expression of PA-related genes across 22 brain regions in a sample of 29 mood-disordered suicide completers and 16 controls identified altered expression of SSAT, S-adenosylmethionine decarboxylase, ODC, antizyme 1 and 2, and arginase II (Fiori et al. 2011). Interestingly up-regulated expression of spermine synthase (SMS) and decreased expression of SMO have also been observed in brains of suicide completers. The altered expression of three important enzymes involved in Spm metabolism, such as SSAT, SMS and SMO, provides a broader view of the nature and level of the deregulation of Spm in suicide and highlights the importance of this molecule in the neurobiology of suicide. SMO activity affects brain physiology and pathology not only altering substrate homeostasis but also by accumulation of the reaction products. The SMO products hydrogen peroxide and 3-AP are both cytotoxic compounds. In particular, H_2O_2 can be source of brain injury and 3-AP spontaneously changes into acrolein, whose high reactivity plays an important role in the development of oxidative cell damage (Zahedi et al. 2010). Recently, increased brain PA catabolism after traumatic brain injury (TBI) has been demonstrated, with concomitant generation of toxic metabolites (H_2O_2 and acrolein) that may contribute to secondary injuries (Zahedi et al. 2010). TBI affects civilian and military populations and in the United States alone nearly 2 million people suffer of TBI each year (Zahedi et al. 2010 and references therein). It is especially important to point out that neurotraumatic brain damage continues to evolve during the hours, days, months and perhaps longer after the mechanical injury. TBI is frequently associated with secondary brain damage caused by hemorrhage, edema, oxidative stress, ischemia, cerebral vascular dysfunction (vasospasm, hyperemia) or thrombosis. Ameliorative treatments would be expected to work either by protecting the brain from the deleterious effects of mechanical and secondary damage or by delaying the onset of secondary injuries (to extend the therapeutic window for more definitive treatments). PA homeostasis is disrupted after brain injuries, with concomitant generation of toxic metabolites by increased PA catabolism that may contribute to secondary injuries. In fact, SSAT mRNA was observed to increase subacutely (6–24 h) after TBI in ipsilateral cortex and hippocampus, while SMO mRNA levels were elevated late, from 3 to 7 days post-injury (Zahedi et al. 2010). The late induction of SMO correlates very well with Spm increase in the ipsilateral injured regions compared to equivalent contralateral regions, suggesting that SMO activity might be elevated at later times post-injury. Thus, Spm oxidation may also be considered a source of secondary tissue damage, increased inflammation and apoptotic cell death in the injured brain. Thus, treatments that retard PA catabolism and reduce cytotoxic oxidative products are expected to reduce secondary tissue damage in brain regions that have enhanced PA catabolism following TBI (Zahedi et al. 2010). In this regard, it is interesting to highlight that a decrease in Spm and an increase in protein-conjugated acrolein (PC-Acro) in plasma is a good biochemical marker for brain infarction. In cells, Spm is mainly metabolized by SMO, and acrolein can be generated during this metabolism (Yoshida et al. 2010). It is also thought that acrolein is more effectively produced from polyamines rather than from phospholipids (Yoshida et al. 2010). However, acrolein is not effectively produced under normal conditions, likely because PA mainly exist as a RNA–polyamine complex and not in the free form (Igarashi and Kashiwagi 2010). It is possible that acrolein is effectively produced when cells are damaged, so that acrolein may become a good biochemical marker for pathologies involving cell damage. The levels of SMO, APAO and PC-Acro in plasma were significantly increased in stroke patients. The size of the stroke was reflected by the multiplied value of PC-Acro and total polyamine oxidases (SMO plus APAO). The results indicate that PC-Acro and polyamine oxidases are good markers of stroke (Igarashi and Kashiwagi 2010). There are also reports indicating that 3-AP, which automatically produces acrolein, is generated from Spm and is strongly involved in cell damage during ischemia in rats (Igarashi and Kashiwagi 2010). The level of PC-Acro is greatly increased, and the levels of Spm and Spd are decreased at the locus of infarction 24 h after the induction of stroke in mice (Igarashi and Kashiwagi 2010). It has been reported that silent brain infarction (SBI) increases the risk of subsequent stroke and dementia and early detection of SBI by biochemical markers is therefore useful for prevention or treatment of stroke (Igarashi and Kashiwagi 2010).

Conclusions

Ten years have passed since SMO was first cloned, and a significant progress has been made in the knowledge of the structure/function relationships of this enzyme. SMO plays

an important role for maintaining the highly regulated PA homeostasis in mammalian cells and the dysregulation of this enzyme has the potential to lead to several pathological conditions, like the inflammation-associated carcinogenesis (Babbar and Casero 2006). It is well recognized that SMO is involved in carcinogenesis, being a source of ROS-induced DNA damage (Amendola et al. 2009; Casero and Pegg 2010), and in breast cancer it represents a negative marker when expressed at low level (Cervelli et al. 2010). SMO can be considered a death inducer agent, and an increasing therapeutic interest has been focused on the interaction between Spm analogs and SMO (Casero and Pegg 2010). The response of SMO to analog treatment likely plays a significant role in determining tumor cell sensitivity to many of the antitumor polyamine analogs (Pledgie et al. 2005). For example, the Spm analog BENSpm is capable to produce cytotoxicity on some human BC cell lines by SMO induction (Pledgie et al. 2005). In this regard, the availability of purified recombinant SMO proteins that allows in vitro biochemical characterization, as well as modeling studies on the enzyme-analog complexes, is crucial to understand if the Spm analogs utilized in therapeutical approaches behave like substrates, thus producing H_2O_2, or inhibitors (Amendola et al. 2009; Cervelli et al. 2010). The SMO substrate Spm plays a dual role in some cells like neurons, since it is involved in PA functions and it can act as a neuromodulator (Masuko et al. 2003; Han et al. 2008). This is in line with data indicating a high level of SMO expression in the brain (Cervelli et al. 2004). It is well demonstrated that Spm has an important role in brain function, modulating the response of several glutamate receptors (kainate, AMPA and NMDA), as well as Kir and Na^+ channels (Williams 1997; Masuko et al. 2003; Han et al. 2008). In recent years, the dysregulation of *SMO* gene expression has been linked to suicidal behaviors and directly associated with psychiatric disorders (Fiori et al. 2010 and references therein). A low level of this enzyme and a high level of SMS have been reported in suicide completers reinforcing the role of Spm in this mental illness (Fiori et al. 2010). For a long time, the therapeutical approach for mental disorder has been mainly focused on the monoaminergic system, nowadays the PA metabolism could represents a new pathway to target for the development of pharmacologic treatments. Recently, increased brain PA catabolism after TBI has been demonstrated, with an increase in SSAT mRNA in the subacute phase and an increase in SMO mRNA in the late phase, indicating a probable role in ROS generation in the secondary brain damage (Zahedi et al. 2010). Thus, potential therapeutic approaches to prevent secondary tissue injury in the treatment of TBI could be directed at increasing tissue PA levels or inhibiting their catabolism. Acroelin is produced from 3-AP by SMO metabolism via Spm oxidation, a decrease in Spm content concomitant to an increase in PC-Acro in plasma is a good biochemical marker for brain infarction (Yoshida et al. 2010). In normal conditions, Spm is not free but mainly exist as a RNA-polyamine complex and not available as substrate for SMO activity. During cell damage, probably, Spm is released from complexes, and the related increase in SMO activity leads to acrolein accumulation (Igarashi and Kashiwagi 2010). Until recently, there were no reliable biomarkers available for the early phase of brain stroke. Thus, these data pave the way to new diagnostic applications of SMO.

In summary, the implication of SMO and Spm in so many basal cellular processes is nowadays well established, but additional work will be required to provide insight into the specific role of this enzyme and its substrate in cancer development and brain functioning.

Acknowledgments The authors wish to thank the University of Roma Tre for financial support.

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