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Genetic Manipulation of Polyamine Catabolism in Rodents

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Activation of polyamine catabolism through the overexpression of spermidine/spermine N^1-acetyltransferase (SSAT) in transgenic rodents does not only lead to distorted tissue polyamine homeostasis, manifested as striking accumulation of putrescine, appearance N^1-acetylspermidine and reduction of tissue spermidine and/or spermine pools, but likewise creates striking phenotypic changes. The latter include loss of hair, lipoatrophy and female infertility. Forced expression of SSAT modulates skin, prostate and intestinal carcinogenesis, induces acute pancreatitis and blocks early liver regeneration. Although many of these features are directly attributable to altered tissue polyamine pools, some of them are more likely related to the greatly accelerated flux of the polyamines caused by activated catabolism and compensatorily enhanced biosynthesis.

Key words: acetyl-CoA, keratinocyte, hairlessness, ornithine decarboxylase, spermidine/spermine N^1-acetyltransferase, transgenic mouse and rat.

Abbreviations: AdoMetDC, *S*-adenosylmethionine decarboxylase; DFMO, 2-difluoromethylornithine; ODC, ornithine decarboxylase; PAO, polyamine oxidase; SMO, spermine oxidase; SSAT, spermidine/spermine N^1-acetyltransferase; TRAMP, transgenic adenocarcinoma of mouse prostate.

Polyamine catabolism in mammals

The biosynthetic reactions leading from L-ornithine to spermine involve two decarboxylation reactions and hence are irreversible. However, experimental results obtained with tracer studies already in the late 1960's indicated that the higher polyamines spermidine and spermine can be converted back to putrescine in rat liver (*1*). Much later it became evident that the conversion of spermine to spermidine and spermidine to putrescine required the concerted action of two different enzymes, a peroxisomal flavoprotein polyamine oxidase (PAO) (*2*) and a cytosolic spermidine/spermine N^1-acetyltransferase (SSAT) (*3*). PAO is a constitutive enzyme, which practically only uses acetylated spermidine and spermine as substrates (*2*) and hence could be more appropriately called acetylpolyamine oxidase. SSAT is highly inducible, turns over extremely rapidly and is the rate-controlling enzyme in the backconversion reactions (*4*). We found that SSAT-deficient mouse embryonic stem cells could not convert spermidine to putrescine, yet the conversion of spermine to spermidine occurred even at an enhanced rate in the targeted cells (*5*). The latter phenomenon was obviously attributable to a recently discovered spermine oxidase (SMO), which specifically catalyzes the oxidation of spermine, but not spermidine or acetylated polyamines (*6*, *7*). Putrescine is terminally oxidized by diamine oxidase, an amine oxidase with limited tissue distribution (*8*).

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Polyamine homeostasis during activated catabolism

As SSAT is the rate-controlling enzyme in the SSAT/PAO-dependent catabolism of spermidine and spermine, its overexpression leads to the activation of the whole catabolic pathway. The activation of polyamine catabolism in mammalian cells and tissues is seen as marked accumulation of putrescine, reduction of spermidine and/or spermine pools, appearance of N^1-acetylspermidine (not normally found in animal tissues) and as a compensatory increases in ornithine decarboxylase (ODC) and *S*-adenosylmethionine decarboxylase (AdoMetDC) activities (*9*). The metabolism of the polyamines has to be considered as a cycle rather than two linear pathways consisting of biosynthesis and catabolism. The polyamine cycle, also called "metabolic ratchet" (*10*), is depicted in Fig. 1 in the form of a paddle wheel. An overexpression of SSAT greatly enhances the overall flux of the polyamines and, by decreasing the pools of spermidine and spermine, induces a compensatory increase in the activities of the two decarboxylases. As indicated in Fig. 1, along with the enhanced SSAT activity, overaccumulated putrescine in all likelihood is the driving force of the wheel. In an attempt to correct the distorted polyamine homeostasis typical to SSAT overexpressing mice, we generated a hybrid transgenic mouse line overexpressing both SSAT and ODC (*11*). To our surprise, the forced expression of ODC did not lead to the replenishment of the reduced spermidine and spermine pools in SSAT mice, but further markedly decreased their content even in the presence of massive putrescine overaccumulation (*11*). The latter phenomenon can be understood in terms that the extra putrescine derived from the enhanced ODC expression, instead of restoring the reduced pools of the higher polyamines, further accelerated the rotation of the wheel (Fig. 1). Such a metabolic overdrive

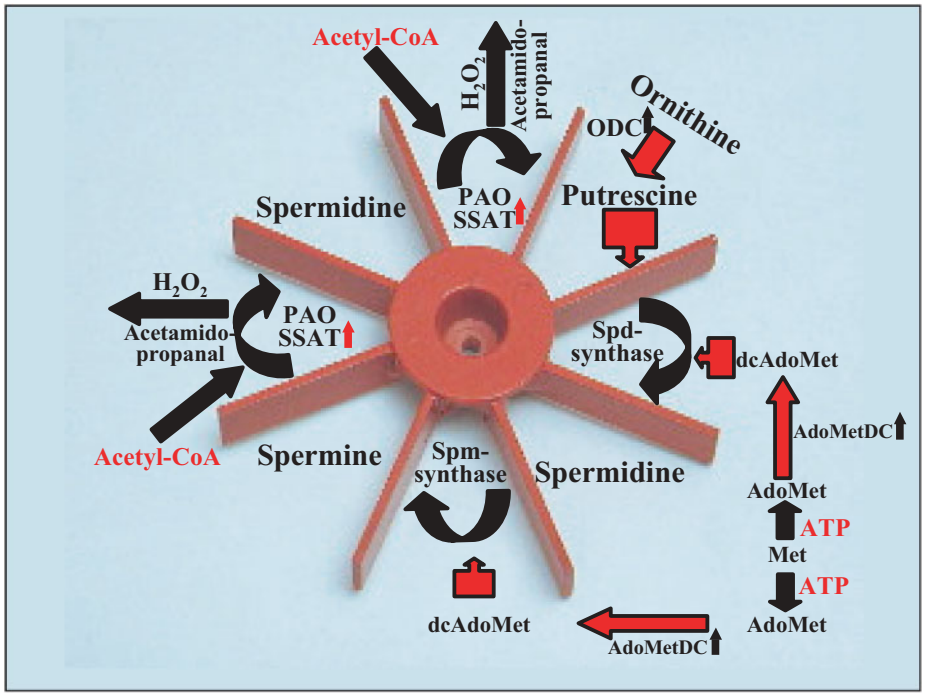

Fig. 1. **"The polyamine paddle wheel."** The enhanced spermidine/spermine N^1-acetyltransferase (SSAT) expression compensatorily induces ornithine decarboxylase (ODC) and *S*-adenosylmethionine decarboxylase (AdoMetDC). The wheel is mainly kept rotating by a continuous supply of putrescine and decarboxylated AdoMet (dcAdoMet). Each round consumes four ATP equivalents. Spd, spermidine; Spm, spermine.

apparently is not a healthy condition, as the hybrid mice have a greatly reduced life-span (*11*). As shown later, cutting off the putrescine supply by an inhibition of ODC in SSAT overexpressing mice slows down the cycle.

Transgenic rodents with genetically altered polyamine catabolism

SSAT has been overexpressed in transgenic mice under its natural promoter (*9*) and in transgenic mice and rats under the heavy metal–inducible metallothionein I promoter (*12*, *13*). SSAT has also been overexpressed in transgenic mice by targeting the expression to outer root sheath keratinocytes with the aid of keratin 6 promoter (*14*). SSAT gene has likewise been disrupted in mice (*10*). An activation of polyamine catabolism through the overexpression of SSAT brings about a plethora of phenotypic changes that will be described in the following in a tissue-specific manner.

Activation of polyamine catabolism profoundly affects skin physiology, causes general lipoatrophy and female infertility

The most prominent phenotypic change in mice overexpressing SSAT was an early and permanent loss of hair apparently caused by replacement of hair follicles by dermal cysts (*9*). Cytokeratin staining indicated that the latter cysts were apparently derived from remnants of hair follicles (*15*). The hairlessness and disturbed keratinocyte differentiation were apparently attributable to strikingly increased skin putrescine concentration. Indirect evidence to support the role of putrescine came from the hybrid mice overexpressing both SSAT and ODC, as these animals with extremely high levels of putrescine in the skin showed significantly more severe skin histopathology than SSAT overexpressing mice (*16*). Similarly, treatment of the SSAT mice with 2-difluoromethyornithine (DFMO), an irreversible inhibitor of ODC, markedly decreased skin putrescine level and induced a distinct hair regrowth (*15*). Further support for the view comes from mice overexpressing ODC under keratin promoter. These animals likewise show identical skin histopathology and lack hair, the loss of which, however, could be prevented by an early administration of DFMO (*17*).

The overexpression of SSAT also appears to modulate skin carcinogenesis, yet in a contradictory fashion. SSAT transgene expressed under its own promoter appears to protect the animals from chemical skin carcinogenesis (*16*), whereas keratin promoter–driven SSAT overexpression rendered the animals more susceptible to tumor development (*14*). This controversy may be related to the fact that the latter animals do not have any skin abnormalities, whereas the former animals lack hair and subcutaneous fat layer. There appears to be a significant positive correlation between the thickness of the dermal fat layer and the development of skin tumors (*18*).

As mentioned, the primary characterization of the SSAT mice revealed that these animals lack subcutaneous fat (*9*). Later we and others (*19*) found that SSAT overexpression also led to the disappearance of visceral fat depots and hence to general lipoatrophy. In contrast to other lipoatrophic animal models (*20*), the SSAT mice did not accumulate fats in non-adipose tissues and, paradoxically, showed enhanced insulin sensitivity (*21*). Their basal metabolic rate was elevated, they showed impaired ketogenesis upon fasting and significantly reduced blood glucose and insulin levels (*21*). These changes may be related to a profound depletion of acetyl-CoA pools at least in some tissues (*19*). The SSAT mice could thus represent metabolically "a reverse model" for type 2 diabetes.

The female members of the SSAT overexpressing mice are infertile due to uterine hypoplasia and lack of corpora lutea (*9*). Although female reproductive organs of the SSAT mice show some differences in gene expression pattern in comparison with wild-type mice (*22*), the contribution of these changes to the infertility remains to be elucidated.

Induction of SSAT in transgenic rats leads to the development of acute pancreatitis and prevents early liver regeneration

Pancreas occupies a very special position among mammalian tissues, as it contains more spermidine and displays higher ratio of spermidine to spermine than any other tissue (*23*). The role of very high pancreatic spermidine content is entirely unknown, but may be related to the extremely active protein synthesis continuously going on in this organ. An exposure of transgenic rats with metallothionein-driven SSAT to zinc rapidly induces SSAT, depletes pancreatic pools of the higher polyamines and causes acute, necrotizing pancreatitis in the transgenic, but not in wild-type, rats (*13*). As indicated in Fig. 1, the concerted action of SSAT and PAO generates hydrogen peroxide and reactive aldehydes, which can be responsible for the pancreatic inflammation. This possibility was, however, excluded, as a specific inhibition of PAO did not prevent zinc-induced pancreatitis, but, if anything, made the situation even worse (*13*). The notion that the development of the pancreatitis was causally related to the profound depletion of the higher polyamines was strongly supported by experimental results indicating that a prior administration of α-methylspermidine, a metabolically stable and functional analogue of spermidine, prevented the zinc-induced pancreatitis (*24*). Methylated spermidine and spermine analogues distinctly alleviated the outcome of the disease and dramatically protected the animals from early death even when administered after the induction of the pancreatitis (*25*). The development of pancreatitis in response to the activation of pancreatic polyamine catabolism is not confined to this transgenic rat model, as transgenic mice similarly develop pancreatitis (*26*) and an activation of polyamine catabolism is associated with the induction of this disease also in other animal models for acute pancreatitis (*25*). It thus appears that sufficient pools of the higher polyamines are required to maintain pancreatic integrity and functional analogues of the polyamines may offer an entirely new approach to treat this life-threatening disease.

An enhanced hepatic accumulation of putrescine and spermidine is a striking feature typical to early rat liver regeneration. In fact, mammalian ODC was first discovered in tissue extracts from regenerating rat liver (*27*, *28*). Pharmacological interventions of polyamine biosynthesis during liver regeneration have, however, yielded conflicting results as regards the requirement of enhanced polyamine accumulation for liver regeneration to occur. Partial hepatectomy of transgenic rats harboring the metallothionein-driven SSAT gene resulted in an immense induction of hepatic SSAT, near-total depletion of the higher polyamines and failure to initiate the regenerative process of the liver (*29*). The view that the profound depletion of the hepatic polyamines was causally related to the prevention of early liver regeneration was convincingly supported by experimental findings indicating that α-methylspermidine fully restored liver regeneration when given prior to the operation (*24*). However, the possibility remains that the impaired liver regeneration is only indirectly related to spermidine depletion as the latter compound serves as the sole precursor for hypusine, an integral part of eukaryotic initiation factor 5A (*30*), which is required for mammalian cell proliferation to occur (*31*). Moreover, methylspermidine can also be converted to hypusine (*32*). A hypusine depletion-based growth inhibition was rendered unlikely by experiments showing that liver regeneration could likewise be restored with dimethylspermine, which is not supposed to give rise to hypusine formation (*33*). These results indicate that a depletion of spermidine and spermine *per se* is responsible for the inhibition of early liver regeneration and that both polyamines may be fully exchangeable in supporting the regenerative growth of the liver.

SSAT expression modulates tumorigenesis in prostate and intestine

As indicated earlier, overexpression of SSAT in the skin either protects transgenic mice from chemical carcinogenesis (*16*) or renders them more susceptible to develop skin papillomas (*17*) depending on the nature of the promoter. SSAT overexpressing mice have been crossed with TRAMP (transgenic adenocarcinoma of mouse prostate) mice. The latter mice harbor SV40 large T antigen driven by prostate-specific and androgen-responsive probasin promoter in their genome rendering them predisposed to develop prostate cancer (*34*). SSAT overexpression in the TRAMP/SSAT hybrid mice was associated with marked suppression of tumor growth (*19*). Even though the activation of polyamine catabolism in the hybrid mice led to a prostatic overaccumulation of putrescine and N^1-acetylspermidine with only marginal changes in the pools of the higher polyamines, the most plausible reason for the suppression of tumor development was a profound (about 70%) reduction of the acetyl-CoA pool and also a substantial decrease in AdoMet content in the prostate of the hybrid mice (*19*). Interestingly, the reduction of acetyl-CoA pool appeared to be tissue-specific, as in the livers of the hybrid mice the decrease in the coenzyme content was much smaller (*19*). This study clearly shows that the pathophysiological consequences resulted from activated polyamine catabolism are not necessarily attributable only to the alterations of tissue polyamine homeostasis but may involve changes in centrally important metabolic pathways due to the greatly accelerated polyamine flux. One may notice from Fig. 1, that each round of the polyamine wheel consumes four ATP equivalents (two molecules of ATP and two acetyl-CoA molecules) and the faster the wheel rotates the more energy is consumed.

SSAT overexpression or lack of expression appears to have an ultimate role also in intestinal tumorigenesis, yet fundamentally differing from that observed in prostatic carcinogenesis. SSAT overexpressing mice have also crossbred with so-called MIN mice. The latter animals carry a truncated adenomatous polyposis coli (*Apc*) tumor suppressor gene and hence are extremely prone to develop multiple intestinal adenomas (*35*). Unexpectedly, the hybrid MIN/

SSAT overexpressing mice developed 3 to 6 times more adenomas in the small intestine and colon than the original MIN mice (*10*). The notion that the level of SSAT expression contributes to the intestinal tumorigenesis was strongly supported by the observations showing that crossing of the MIN mice with mice carrying targeted disruption of the SSAT gene reduced the incidence of adenomas in the small intestine by 75% (*10*). It thus appears that SSAT overexpression can tissue-specifically modulate tumorigenesis in an entirely opposite fashion. Neither hybrid mice showed any marked alterations of pools of spermidine and spermine obviously attributable to compensatory increases or decreases in the activities of the biosynthetic decarboxylases (*10*). Once again, one has to consider the contribution of the altered polyamine flux rather than the possible changes in polyamine tissue pools. It is highly possible that the accelerated polyamine flux, as resulted from SSAT overexpression and compensatory increases in the biosynthetic decarboxylases, could facilitate tumorigenesis through the enhanced generation of reactive oxygen species by the polyamine cycle that could in turn contribute to the loss of heterozygosity at the *Apc* locus, the ultimate oncogenic mechanism in this model of tumorigenesis (*10*).

Consequences of the activation of polyamine catabolism in central nervous system

The role of enhanced accumulation of polyamines, especially putrescine, in the context of various chemical and physical brain insults has been a subject of controversy for a few decades. The most widely accepted view appears to associate putrescine overaccumulation in central nervous system with the development of neuronal injury rather than a neuroprotective procedure (*36*). Brain insults, almost without an exception, lead to an induction of ODC and a subsequent accumulation of brain putrescine with little changes in the pools of spermidine and spermine (*37*). Similarly, some neurotoxins have been reported to enhance brain SSAT activity (*38*). Our experiments with SSAT overexpressing mice strongly support the notion that an enhanced putrescine accumulation in the brain offers a distinct neuroprotection to the animals. This is exemplified by the experiments indicating that SSAT overexpression and enhanced putrescine accumulation in the brain protects the transgenic animals from kainate-induced toxicity by greatly reducing the overall mortality and neuronal damage (*39*). Similarly, SSAT transgenic mice showed significantly elevated threshold to pentylentetrazol-induced convulsions (*40*). The results of a neurobehavioral profiling of SSAT mice indicated that the transgenic animals are hypomotoric, less aggressive and have spatial learning impairment (*41*). Taking together these results as well as those obtained with ODC overexpressing mice and rats (*37*), we have reached the conclusion that very high brain putrescine concentrations and a strikingly elevated molar ratio of putrescine to the higher polyamines will lead to a partial blockade of the *N*-methyl-D-aspartate receptor, for which spermidine and spermine function as receptor agonists (*42*).

Concluding remarks

The activation of polyamine catabolism in transgenic rodents brings about a number of bizarre phenotypic changes, of which many, but certainly not all, are directly attributable to altered tissue polyamine pools as a result of SSAT overexpression. The latter apparently include disturbed keratinocyte differentiation ultimately leading to loss of hair and neuronal protection caused by the overaccumulation of putrescine in the skin and brain. Similarly, profoundly depleted pools of spermidine and spermine in the pancreas and liver as a result of SSAT induction are in all likelihood causally related to the development of acute pancreatitis and inhibition of liver regeneration. However, the observed modulation of tumorigenesis by SSAT expression is apparently not attributable to the altered tissue polyamine pools but are related to the greatly enhanced polyamine flux due to SSAT overexpression and compensatorily increased biosynthesis. The accelerated polyamine flux (Fig. 1) leads to the consumption of centrally important metabolites, such as acetyl-CoA and ATP, and generates potentially harmful compounds, such as hydrogen peroxide and reactive aldehydes. Accordingly, inhibition or facilitation of tumorigenesis by SSAT expression may be tissue-specific depending on the ambient metabolic environment. In any event, all the observed phenotypic changes are in one way or another related to the altered metabolism of the polyamines.

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