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MOLECULAR REGULATION OF ORNITHINE DECARBOXYLASE (ODC) ENZYME IN MAMMALIAN CELLS

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ABSTRACT

Polyamines (putrescine, spermidine and spermine) are ubiquitous low molecular weight amines that are positively charged under physiological conditions. Homeostatic control of intracellular polyamines levels is achieved by regulating the synthesis, catabolism and transport of these molecules. Polyamines are involved in the regulation of diverse range of vital cellular processes in both eukaryotic and prokaryotic cells, including cell proliferation, signal transduction and membrane stabilization. Putrescine, spermine and spermidine, universally occurring in mammalian cells, are involved in a wide array of processes, ranging from triggering growth and proliferation to protecting against stress. Ornithine decarboxylase (ODC) initiates the polyamine biosynthetic pathway by producing putrescine. The amount of ODC is altered in response to many growth factors, oncogenes and tumor promoters and to changes in polyamine levels. This review describes key factors that contribute to the regulation of ODC levels and activities, which can occur at the levels of transcription, translation and protein turnover.

KEYWORDS: *polyamines, ornithine decarboxylase, antizyme, antizyme inhibitor, cancer.*

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INTRODUCTION

Mammalian cells evolved throughout the time, gaining many regulatory molecules to synchronize their growth and proliferation. One of the regulatory molecular groups known is the polyamines. These are naturally occurring major amines which have biological activities inside and outside the cells. The group including putrescine, spermidine, spermine and their metabolites such as N-acetyl spermidine and N-acetyl spermine. The three major polyamines in mammalian cells are synthesized in sequence from ornithine to putrescine to spermidine to spermine.[1] Each of them is small, straight chain aliphatic water soluble carbon-nitrogen molecules with the amino groups evenly distributed throughout.[1-2] The rate limiting step of polyamine synthesis is decarboxylation of ornithine to form putrescine by ornithine decarboxylase (ODC; EC 4.1.1.17).[3-4] This is followed by decarboxylation of S-adenosylmethionine to decarboxylated S-adenosylmethionine by the enzyme S-adenosylmethionine decarboxylase (SAMDC; EC 4.1.1.50). Decarboxylated S-adenosylmethionine is then combined with putrescine to produce spermidine, an enzymatic step catalyzed by spermidine synthetase (EC 2.5.1.16).[4-7] Another decarboxylated S-adenosylmethionine is then added to spermidine by spermine synthetase (EC 2.5.1.22) to produce spermine. The spermidine and spermine can be catabolized by one specific enzyme, spermidine/spermine N1-acetyltransferase (SSAT; EC2.3.1.57) to become N-acetyl-spermidine and N-acetyl-spermine, respectively.[4-6] The N1-acetyl polyamines are the preferred substrates of FAD-dependent polyamine oxidase (EC1.5.3.11), which convert N-acetyl-spermidine to putrescine, and N-acetyl-spermine to spermidine, respectively.[1-7] Although the precise physiological functions of these amines have not been defined, polyamines have been proved to be essential for growth, proliferation and differentiation of virtually all eukaryotic and prokaryotic cells. As such, increases in polyamine biosynthesis via the regulation of ODC usually accompany the increases in cell growth in response to hormones and growth factors.[2-3] Figure 1 depicts the metabolism of polyamines in mammalian cells.

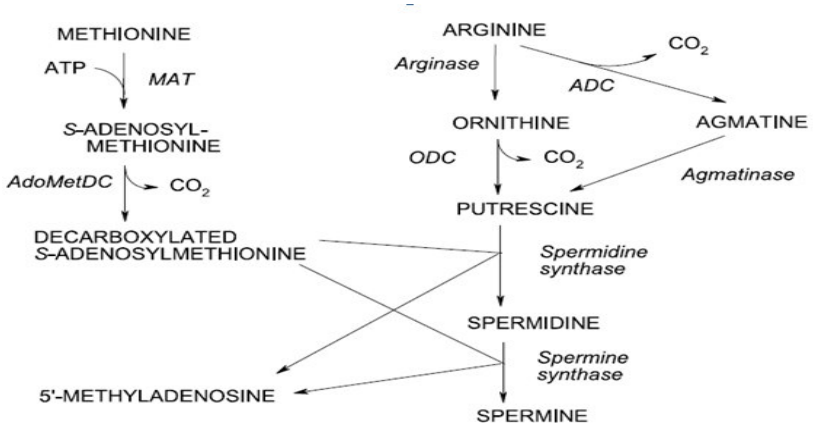

Figure 1
Mammalian polyamine biosynthetic pathway

ODC ENZYME REGULATION

ODC is a dimeric enzyme with several novel regulatory features. It is a highly inducible, cytosolic, subunit enzyme that responds to a range of trophic stimuli.[8] It has a short half-life (10 minutes to 1 hour) compared to many mammalian enzymes whose half-lives are more often expressed in days.[9] ODC requires pyridoxal phosphate as a cofactor and thiol-group reducing agents are necessary for enzyme activity, possibly owing to the high number of cysteine residues in the protein. ODC contains two PEST (proline-, glutamate-, serine- and threonine-rich) regions that are also rich in proline, glutamic acid, aspartic acid, serine and threonine.[10] The PEST region located at the C-terminus of ODC is essential for degradation of the enzyme, and truncations and mutations in this region result in destabilization of the enzyme.[11] ODC activity is dependent upon formation of a dimer with the active site, occurring at the interface between the two subunits.[12] Residues at the active site that are critical to ODC activity including Lys169 and His197.[13] ODC expression is also regulated by transcription, stability and the efficiency of mRNA translation. At a transcriptional level, ODC expression can be regulated by oncogenes. The hODC gene contains three CACGTG regions: one at the 5 promoter region and the two others in intron 1 that bind the protein product of the c-Myc oncogene.[14-15] Overexpression of c-Myc and other oncogenes such as v-Mos, Ha-ras and c-Fos [16-18] can lead to overexpression and induction of ODC and, ultimately, carcinogenesis. ODC mRNA has long 5 and 3 untranslated regions (UTRs) and neither region seems to be involved in polyamine-mediated feedback control of ODC activity.[19] The 3UTR may have a role in regulation under special circumstances, such as hypotonic shock.[20] Increases in ODC activity are one of the early changes observed in cells stimulated to grow and these increases precede changes in DNA synthesis by several hours, making ODC as an 'immediate early'-response gene.[21] ODC is subjected to both positive and negative feedback regulation by polyamines, including high polyamine concentrations decrease, and low polyamine concentrations increase activity. The feedback regulation appears to be a mixture of post-

transcriptional regulation and the induction of a unique ODC-specific inhibitor termed 'antizyme' (AZ).[22]

REGULATION OF ODC BY ANTIZYMES

The family of AZ functions as regulators of polyamine homeostasis.[23] They are a class of small, inhibitory proteins, whose expression is regulated by a unique ribosomal frameshift mechanism. They have been shown to inhibit cell proliferation and possess anti-tumor activity. AZ binds ornithine decarboxylase (ODC).[24-25] They inhibit its enzymatic activity and promote the ubiquitin-independent degradation of ODC by the 26S proteasome.[25] In addition, they also negatively regulate polyamine transport. AZ-mediated, ubiquitin-independent degradation of ODC is conserved from yeast to man. But recent data suggest that this degradation pathway might not be restricted to ODC alone and could involve newly discovered AZ binding partners.[23-25] Interestingly, AZ proteins have been strictly preserved over a vast evolutionary timeframe. AZ consequently represents an important class of proteins that regulate cell growth and metabolism by a diverse set of mechanisms that include protein degradation, inhibition of enzyme activity, small molecule transport and other, potentially not yet discovered properties. AZ sequesters monomeric ODC molecules. AZ prevents the dimerization and formation of enzymatically active ODC.[23] Binding of AZ leads to conformational changes in ODC and exposure of the C-terminal PEST sequence that provokes translocation to the 26S proteasome for degradation without ubiquitination.[24] AZ itself is not degraded together with ODC, but is recycled back to the cytoplasm. Due to antizyme-induced degradation, the half-life of ODC (10-20 minutes) is among the shortest known for proteins in mammalian cells.[23,24] An increased polyamine content protects AZ from ubiquitin-mediated degradation and enhances AZ expression by affecting the rate of ribosomal frame-shifting.[25] AZ family comprise of three to four members in mammalian, with AZ1 as the most prominent member. AZ1 promotes degradation of ODC via the 26S proteasomes in an ubiquitin-independent manner. AZ1 also contains a mitochondrial targeting motif. It is transported to the mitochondrial membrane where it depolarizes the membrane and activates the caspase cascade leading to the induction of apoptosis.[26] Over-expression of AZ1 inhibits cell proliferation and growth via ODC inhibition and reduction of the polyamine content.[23-24] AZ can be considered as a tumor suppressor. Depletion of AZ in cultured cells leads to over-duplication of centrosomes, whereas the silencing of antizyme inhibitor (AZI) reduces centrosome abnormalities.[27] These data suggest that AZ and AZI are connected to the early stages of carcinogenesis in which the loss of tumor suppressors triggers defects in centrosome functioning. Recent data suggests that AZ is not solely a regulator of ODC but also controls the degradation of other proteins that participate in growth regulation, e.g. cyclin D1, Smad1 and Aurora-A.[28] Antizyme inhibitors (AZIs) have arisen from ODC by gene duplication and thus share a high degree of sequence similarity to ODC.[23-24] Due to the homology, AZIs bind AZs, with even higher affinity than ODC, and thus liberate ODC from the heterodimer complex with AZ resulting in the formation of active homodimers and increased ODC activity.[27] The binding of AZI to AZ also blocks the inhibition of polyamine transporters mediated by AZ, and the uptake of polyamines is enhanced.[28] The binding of AZ to AZI or ODC is reversible, and the equilibrium is constantly monitored and adjusted by the concentration of polyamines. In biochemical assays, AZI binds all known AZs, AZ1-3.[29] AZI remains a monomer under physiological conditions and it is unable to bind the cofactor, pyridoxal-L-phosphate, which is needed for the enzymatic activity of ODC.[30] The transcription of AZI is increased by growth stimuli, prior to induction of ODC. During the cell cycle, AZI is activated similarly to ODC in late G1 and again in G2/M, and during mitosis (M) it is located in the centrosome analogously to AZ.[31] The growth-promoting activity of AZI may not be solely dependent on the neutralization of AZ, since AZI has been demonstrated to stabilize cyclin D1 independently of the AZ-binding. The expression of AZI1 is elevated in human gastric cancer compared to normal gastric tissue as well as in Ras-transformed fibroblasts.[32] AZI also promotes the survival of various types of cancer cells via activation of ODC under hypoxic conditions.[33] In carcinogenesis, activation of ODC is considered to be an early step in malignant transformation. It has, however, been postulated that the activation of ODC might actually proceed via induction by AZI.[34] ODC activation itself leads to a rapid increase in the amount of AZ, leading to the reciprocal diminution of ODC activity and polyamine uptake. In contrast, the activation of AZI blocks AZ and promotes cell growth via sustained polyamine accumulation.

LOCALIZATION OF ODC

Intracellular localization of ODC and polyamines is functionally of great importance, since polyamines participate simultaneously in various cellular functions in the nucleus, mitochondria, plasma membrane, secretory vesicles and cytoplasm.[1] Investigations on the localization of ODC have proven difficult due to its extremely short half-life, and thus create minute amount of detectable protein. On the other hand, problems have been encountered in determining the compartmentalization of polyamines with the larger pool of bound polyamines compared to freely recruitable ones.[2-3] Polyamines take part in the modulation of various cellular signalling cascades for which they are either synthesized *de novo* or transported into extracellular spaces.[2-3,5-7] Polyamines bound to nucleic acids and proteins are considered rather inactive in the event of the rapid recruitment for signalling. Mitogenic signalling translocates ODC to the nucleus,[1] possibly in connection with AZ that is considered to regulate the nucleocytoplasmic shuttling of ODC.[4] Indeed, AZ contains two nuclear export signals.[1,4] Immunochemical stainings from different cell lines indicate that AZ is mainly localized to the nucleus in actively proliferating cells. Epitope-tagged AZI1 has also been detected in the nucleus of proliferating, cultured cells suggesting that reciprocal activities of AZ and AZI1 mediate the fluctuations in ODC activity during the cell cycle.[34] However, the role of ODC in the synthesis of nuclear polyamines needs to be further investigated, since the presence of other enzymes needed for polyamine

synthesis have not been described for the nucleus. If the function of nuclear proteasomes is restricted, ODC will accumulate in the nucleus, indicating that ODC degradation targeted by AZ also occurs in the nucleus.[4] AZ might have a more potent role in the degradational targeting in the nucleus, since AZ also mediates the degradation of the oncogene Aurora-A, a gene that is related to the progression of mitosis.[28] During transition from prophase to telophase in mitosis, the proportions of AZ and AZI1 are located in the centrosomes where they facilitate the completion of mitosis.[31] Overactivity of AZ leads to a decrease in the number of centrosomes, whereas the increased activity of AZI1 is followed by an accumulation of excess centrioles.[27] After mitosis, the entire orchestra of polyamine regulators, ODC, AZ1 and AZI1 is detected in the perinuclear space.[27] The induction of apoptosis is accompanied by an increase in ODC activity, and the resulting accumulation of putrescine is assumed to contribute to the activation of apoptotic signalling cascades. However, polyamines have also been proposed to play an antiapoptotic role.[1,4] Conversely, the inhibition of ODC by difluoromethylornithine (DFMO) and apoptosis were initiated by both extrinsic (receptor-induced) and intrinsic (mitochondria-derived) pathways.[4] Although ODC itself has not been detected in mitochondria, AZ1 contains an N-terminal motif for mitochondrial targeting. An overexpression of AZ1 in hematopoietic cells leads to its accumulation in mitochondria, which is subsequently followed by caspase cascade- and cytochrome c-mediated apoptosis.[26] Apoptosis, in which partly overlapping in signaling cascades with growth induction are activated, exemplifies the necessity of compartmentalization and localized regulation of polyamines and the regulators of their synthesis.

FUTURE PERSPECTIVE

Over the past 40 years, much progress has been made in understanding the role of the polyamine pathway and ODC activity in normal cell functioning and carcinogenesis. The knowledge gained is now being used to develop new strategies for the treatment and prevention of cancer. Targeting the polyamine biosynthetic pathway for antitumor therapy started soon after the discovery that normal upregulation of polyamine levels were a hallmark for numerous tumor types. Thus, the original therapies that targeted the polyamine pathway were for the metabolic enzymes ODC and S-adenosylmethionine decarboxylase. Difluoromethylornithine (DFMO), an irreversible inhibitor of ODC, was first shown in the 1970s to have antitumor properties. Unfortunately, DFMO, alone or in combination with other agents, was largely ineffective as a chemotherapeutic agent. The lack of effectiveness of DFMO as a chemotherapeutic agent is likely due to its poor transport into the cell, the fact that it is typically cytostatic and not cytotoxic, and compensatory mechanisms such as increased polyamine transport or upregulation of S-adenosylmethionine decarboxylase that occur as a result of the depleted polyamine pools. Thus, tumor cells can overcome the effects of ODC inhibition. One important area in the polyamine field that continues to be poorly understood is the polyamine transport system, influx and efflux. Very little is currently known regarding the molecular components of the mammalian polyamine transport system. To fully exploit the polyamine pathway and to optimize the use of polyamine analogues in a therapeutic setting will require greater knowledge in this area. The polyamine transport system has been used for cellular entry of molecules that are conjugated to a polyamine backbone, however, a greater understanding of the transporter will be necessary to fully exploit this strategy. As the field moves forward and we gain a better understanding of the roles that polyamines play in growth and differentiation in the normal setting, as well as their dysregulation in neoplastic disease, it is likely that more rational targets and better agents to target them will be discovered. There is no doubt that the newly generated animal models, along with a continuing stream of polyamine-based compounds will aid in this endeavor.

CONCLUSION

Ornithine decarboxylase (ODC) initiates the polyamine biosynthetic pathway. The amount of ODC is altered in response to many growth factors, oncogenes and tumor promoters and to changes in polyamine levels. ODC is very highly regulated and ODC activity varies in response to many stimuli. These alterations in activity are brought about by changes in the amount of ODC protein, which turns over rapidly. ODC degradation is controlled by a protein termed antizyme, which responds to polyamine concentration. ODC is also regulated at the level of transcription and ODC gene is one of the targets of Myc/Max transcription factor. The third level of regulation occurs in the translation of ODC mRNA. This review describes key factors that contribute to the regulation of ODC levels, which can occur at the levels of transcription, translation and protein turnover.

CONFLICT OF INTEREST

Conflict of interest declared none.

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