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Minireview

Differential role of polyamines in hyperplasia and hypertrophy

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Hypertrophic *versus* hyperplastic growth

Tissues and organs can respond to a variety of physiological and nonphysiological stimuli by increasing their weight, volume and size. Depending on the kind of stimulus and tissue, organ growth can be achieved by two highly regulated and fundamentally different processes, namely by increase in size of existing cells (hypertrophy), or by cell proliferation (hyperplasia). The major differences between the processes of hypertrophy and hyperplasia are listed in Table 1. The primary difference is the large increase in DNA synthesis associated exclusively with hyperplasia. Consistent with the low levels of DNA synthesis in hypertrophy is only a slight increase of H4 histone gene expression which is known to be tightly coupled to DNA synthesis [1]. Patterns of expression of several proto-oncogenes vary greatly between hypertrophy and hyperplasia; this strongly suggests that proto-oncogenes are primarily involved in the replicative component of growth rather than in hypertrophy stage [1, 2].

An increase in cell size and protein content is characteristic for cells undergoing hypertrophy, but also for replicating cells prior to DNA synthesis. Different gene expression found at the early stages of cell enlargement in hypertrophy and hyperplasia suggests that the two processes are uncoupled, and regulated by different early events [2]. Thus, cell hypertrophy is not due to interruption of the normal cell cycle at a stage of cell enlargement with an increased complement of organelles and molecules (G_1). Cells undergoing hypertrophy do not pass through G_1, but remain quiescent in G_0 in a state of "sustained message amplification" [2].

Table 2 contains several examples of organs and tissues undergoing hypertrophy or hyperplasia under the influence of different stimuli. Thus, in response to partial destruction of the organ, compensatory growth occurs, as in the case of liver regeneration after partial hepatectomy, adaptive intestinal hyperplasia after jejunectomy, or renal compensatory hypertrophy after contralateral kidney removal. Enlargement of the organ, occurring as a part of functional adaptive responses, can also be caused by, among other things, metabolic signals (e.g. hormones), chemical or mechanical tissue injury, or in the case of heart, by strenous physical activity and exercise (Table 2).

Polyamine biosynthesis stimulation in hypertrophy and hyperplasia

Two polyamines, spermidine and spermine, and their precursor diamine putrescine, omnipresent components of all living cells, are aliphatic polycations with three, four and two positive charges, respectively, at physiological pH. In the cell they are bound to macromolecular anionic sites in nucleic acids, ribosomes and membranes. Polyamines are believed to be es-

[1]Abbreviations: ODC, ornithine decarboxylase; AdoMetDC, *S*-adenosylmethionine decarboxylase; SAT, spermidine/spermine N^1-acetyltransferase; DFMO, α-difluoromethylornithine

Table 1
Hypertrophy versus hyperplasia

	Hypertrophy	Hyperplasia
Organ size and weight	increased	increased
Cell size	increased	changable in cell cycle
RNA and protein synthesis	increased	increased
Cell proliferation	not affected	increased
DNA synthesis	not affected	increased
Protein/DNA ratio	increased	unchanged
RNA/DNA ratio	increased	unchanged
Gene expression:		
H4 histone [1]	slightly increased	9-fold increase
Proto-oncogenes (c-*myc*, c-H-*ras*, c-K-*ras*, c-*fos*) [1, 2]	slightly increased	2 - 20-fold increase

Table 2
Hypertrophy or hyperplasia of organs and tissues induced by various stimuli

Organ/Tissue	Stimulus	Hypertrophy/Hyperplasia
Kidney	unilateral nephrectomy	compensatory hypertrophy [1 - 5]
	testosterone	hypertrophy [6 - 10]
	pregnancy	hypertrophy [11]
	folate	hyperplasia [2, 12]
	antifolate CB3717	hyperplasia [10, 13]
Liver	partial hepatectomy	hyperplasia (e.g. [1, 14])
	chemical injury, e.g. carbon tetrachloride	hyperplasia [15]
	pregnancy	hyperplasia [11]
Heart	thyroxine	hypertrophy [16]
	isoproterenol	hypertrophy/hyperplasia [17 - 20]
Aortas	mechanical injury	hyperplasia [21]
Intestine	jejunectomy	hyperplasia [22, 23]
	injury induced by chemotherapy	hyperplasia [24]
	phytohemagglutinin	hyperplasia [25]

sential for cellular growth, proliferation and differentiation, although their physiological function at the molecular level is still not well understood [26, 27]. Polyamine metabolism is stimulated at the onset of hypertrophic and hyperplastic growth processes in the adult organism, independently of applied stimuli, as was shown in the experimental models of hypertrophy and hyperplasia given in Table 2. The most remarkable change appears to be the induction of ornithine decarboxylase (ODC)[1], the first and rate-limiting enzyme in the polyamine biosynthetic pathway. The activity of ODC, responsive to multiple stimuli, increases both in hypertrophy and hyperplasia, although often to differing degrees. In comparative studies on polyamine metabolism in testosterone-induced hypertrophic and antifo-

late-induced hyperplastic mouse kidney models, we found a dramatic, several hundred-fold increase of renal ODC by testosterone, and a much lower increase evoked by antifolate [9, 10, 13]. Interestingly, accumulation of the ODC product, putrescine, was comparable in hypertrophic and hyperplastic kidney, pointing to a precise regulation of its intracellular pool which, similar to other polyamines, can be accomplished by synthesis, interconversion, uptake, degradation and/or covalent incorporation into proteins [10].

In contrast to putrescine levels which increase up to 10-fold in hypertrophic or hyperplastic tissues, spermidine and spermine, which are synthesized later in the polyamine pathway, are always less affected [6, 7, 10, 16, 18, 21, 22].

S-Adenosylmethionine decarboxylase (AdoMetDC), another key regulatory enzyme of polyamine biosynthesis, responded differentially to the growth-promoting stimuli. In the mouse kidney, in contrast to ODC, it was unresponsive to testosterone [9], but its activity increased several-fold in antifolate-induced hyperplastic kidneys [10] and hypertrophic heart [16].

Spermidine/spermine N^1-acetyltransferase (SAT), the enzyme regulating the activity of the polyamine interconversion pathway, was induced in hyperplastic rat kidney [12]. Accumulation of SAT product, N^1-acetylspermidine, found in antifolate-induced hyperplastic kidney [10] and isoproterenal-induced hypertrophic heart [19], provided additional indirect evidence of SAT induction in these two hyperplastic organs.

The response of two key enzymes of the polyamine biosynthetic pathway to hypertrophy and hyperplasia, and polyamine pools are summarized in Table 3.

Functional role of polyamines in hypertrophy and hyperplasia; inhibitor studies

Although a number of studies was aimed at understanding the role of polyamines in regulated organ growth, it was not possible to directly evaluate their critical role until specific inhibitors of their metabolism were developed. A powerful, irreversible and specific inhibitor of ODC, α-difluoromethylornithine (DFMO), introduced in the late 1970's, is the best known and most often used inhibitor in polyamine research (e.g. [26]).

In accordance with the antiproliferative effect of DFMO, hyperplastic organs responded to this inhibitor. Thus, increases in ODC activity and polyamine biosynthesis which have been reported to be critical for recovery from chemotherapy-induced injury of rat intestinal mucosa [24] and adaptive post-resectional intestinal rat hyperplasia [22] were abolished by DFMO. Similarly, in diabetic rats DFMO lowered ODC activity and polyamine content, preventing intestinal epithelium hyperplasia [23]. In a hyperplastic antifolate-induced kidney model, DFMO significantly lowered putrescine and spermidine levels and influenced biochemical markers of hyperplasia, pointing to the role of spermidine (rather than putrescine) in mediation of renal hyperplastic growth [10]. Similarly, spermidine (but not putrescine or spermine) was suggested to be responsible

Table 3
Polyamine metabolism in testosterone-induced hypertrophic and antifolate-induced hyperplastic mouse kidney [10]

	Hypertrophic kidney	Hyperplastic kidney
Ornithine decarboxylase	induced (700 ×)	induced (68 ×)
AdoMet decarboxylase	not affected	induced (4.3 ×)
Polyamine level:		
Putrescine	increased (5.3 ×)	increased (7.8 ×)
Spermidine	increased (1.6 ×)	increased (1.5 ×)
Spermine	insignificantly increased	decreased (0.8 ×)
Spermidine/Spermine ratio	unchanged (< 1)	increased (> 1)
N^1-acetylspermidine	not affected	increased (14.7 ×)

for the intestinal epithelial hyperplasia in diabetic rats [23].

The role of polyamines in the process of hypertrophic growth was studied mainly in renal and cardiac hypertrophy models. Androgen-induced hypertrophic kidney is characterized by spectacular induction of ODC and putrescine content, and a less significant increase of spermidine and spermine [6, 7, 10]. DFMO, by inhibiting ODC, prevented augmentation of putrescine levels, but did not influence kidney hypertrophy [7, 10], biochemical markers of hypertrophy [10], or the response of marker gene to androgen [7]. These findings argue against the previously suggested mediation of androgen response by putrescine in mouse kidney [6]. Similarly, stimulation of renal ODC activity and putrescine content did not appear critical to the process of compensatory renal hypertrophy after unilateral nephrectomy in the rat [3].

Cardiac hypertrophy induced by thyroxine in rats was accompanied by increased activity of polyamine synthesizing enzymes and elevated content of polyamines [16]. This increase was not, however, an obligatory component of the hypertrophic response because thyroxine-induced hypertrophy was not abolished by a non-physiological polyamine [16] or DFMO [28] which prevented the increase in polyamine concentration. In contrast, isoproterenol-induced cardiac growth was attenuated by DFMO, indicating that there are major differences in the cellular mechanisms by which thyroxine and catecholamines elicit increased cardiac growth [28]. In this context it is worth mentioning that DFMO normalized increased kidney weight in experimental diabetes, but only in the second, hyperplastic phase of kidney growth; DFMO had no effect during the initial phase which was due primarily to hypertrophy [29].

Polyamine depletion *in vivo*

The studies on functional coupling between polyamine levels and hypertrophy or hyperplasia are often difficult to interpret due to the fact that even such a specific and potent inhibitor as DFMO produces states of only partial polyamine depletion (significant reduction of intracellular putrescine pool, much lower depletion of spermidine, and negligible reduction or an increase of spermine (e.g.[10]). Moreover, most polyamines which are bound to cell constituents are dispensable in the cell, and cellular growth is not limited until a very small minimum of polyamines is reached [30]. Therefore, it is not surprising that, especially *in vivo*, the effect of DFMO is not always evident.

It appears that blocking only the biosynthetic route is insufficient to deplete cellular spermidine and spermine since the use of DFMO alone triggers compensatory cellular mechanisms to bypass polyamine deprivation, e.g. diminished urinary polyamine excretion [31] or enhanced polyamine transport [32]. In this respect, it is important to remember that food is an abundant source of polyamines, and food polyamines are directed preferentially to tissues and organs that have been stimulated to grow [25]. More significant depletion of polyamines could be achieved by combined treatment of the animal with two or more specific inhibitors of the putrescine, spermidine and spermine biosynthetic and polyamine interconversion pathways [33, 34].

Protein-bound polyamines and transglutaminase in regulated organ growth

Recently a novel aspect of polyamine metabolism has been reported [35, 36]. Polyamines within the cell can be not only bound to cellular anions by electrostatic forces, but can also be irreversibly incorporated into proteins in transglutaminase-dependent reactions, and participate in protein cross-linking. There is evidence that transglutaminase and protein cross-linking are involved in regulation of hypertrophic and hyperplastic growth. High transglutaminase activity occurring in the kidney during anatomical and functional hypertrophy can lead to the formation of putrescine-protein complexes as evidenced in pregnant rats [11]. The repair of gastric and duodenal stress erosions is accompanied by an increase in ODC and transglutaminase activity [37]. Inhibition of mucosal repair by DFMO demonstrated that polyamines are absolutely required for this process. Spermidine prevented inhibition of repair caused by DFMO, but only under conditions of active transglutaminase. Thus, the increased transglutaminase activity and protein cross-linking may be part of the mechanism requiring polyamines for healing of mucosal stress erosions [37].

Conclusions

The presented studies document that the increase of ornithine decarboxylase activity and polyamine content is associated with the onset of both hyperplasia and hypertrophy. However, stimulation of polyamine biosynthesis is critical exclusively for the hyperplastic growth processes in which polyamines serve a mediatory role. Activation of ODC and increase in polyamine content, although accompanying events in hypertrophy, seem not to be obligatory, and often can be dissociated from hypertrophic growth. It is possible that enhancement of polyamine synthesis is not required for hypertrophy, and it is merely a consequence of a change in the rate of protein synthesis and degradation in the growing tissue [16].

Conflicting results on the role of polyamines in hypertrophy can be connected with the fact that it is practically impossible to obtain models of pure hypertrophic growth; usually proliferation accounts to differing degrees for organ growth, depending on the applied stimuli or even animal age [5].

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