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Perspective

Is Cell Size Important?

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ABSTRACT

Cell size plays an indirect role in cell proliferation, as cells must double in size before dividing. Cell size is largely determined by the activity of RNA polymerase I that controls ribosomal RNA synthesis and ribosome biogenesis. The type 1 insulin-like growth factor receptor (IGF-IR) and its docking protein, insulin receptor substrate-1 (IRS-1) control, in a non-redundant way, about 50% of cell and body size. This is certainly true in mice, flies and cells in culture, but also probably in higher mammals. Interestingly, the insulin receptor (InR) cannot substitute for the IGF-IR in controlling cell size. This is probably due to the fact that the IGF-IR is more effective than the InR in translocationg to the nuclei IRS-1, which then binds UBF1, one of the proteins that regulate RNA pol I activity.

INTRODUCTION

Cell size or cell growth? For the purposes of this brief review, cell size is defined as cell mass. There are many investigators who prefer the expression "cell growth" (see for instance Hall et al., ref. 1), as opposed to cell proliferation. Cell growth would then define only an increase in cell mass, exclusive of cell division that would instead be defined as cell proliferation, i.e., increase in cell number. Increase in cell mass and increase in cell number are the components of organismic growth and determine, for instance, the size of an animal. Both components are known to occur in development from the single fertilized egg (self evident) to the adult animal. Although growth is probably the better term, I will use in this review the term cell size, of easier comprehension.

What determines cell size? The answer to this question is very simple if one is content with an iterative solution. Cell size is given by the amounts of proteins and ribosomal RNA (rRNA) per cell. Proteins constitute most of the dry mass of a cell, and the amount of protein/cell is influenced by the number of ribosomes, i.e., the amount of rRNA.[2] Therefore, cell size is determined by ribosome biogenesis,[3] which depends largely on ribosomal RNA (rRNA) synthesis.[4] In turn, rRNA synthesis is under the control of RNA polymerase I and the proteins that associate with RNA pol I in the rDNA promoter.[5] In other words, cell size is controlled by RNA pol I activity. This is a pretty good approximation, a real iterative solution, i.e., a solution that is not accurate but is sufficient for the task at hand (the first two decimals of π are sufficient to build a jet engine or the wheels of a car). If you do not like iterative solutions, cell size is also dependent on protein turnover, a slow turnover of proteins leading to protein accumulation. It is also dependent on nutrient availability, something again self evident (try to make cells grow if you do not provide them with the essential amino acids.). This explains the importance of mTOR in determining cell size, as mTOR can be activated by growth factors and by nutrients. Thus, mTOR is at the center of different pathways, one of which is dependent on PI3-K (see below).

Cells come in all sizes. Growth in size is a requirement for cell division (see next section). When I mention this (to me) self-evident conclusion, I often hear the objection that small cells can grow as fast as big cells. That is absolutely correct. The size of the cell, for any given cell type, is unrelated to the speed of cell proliferation. But each cell type, whether in the living animal or in tissue cultures, has an optimal size, which has to be doubled before cell division, so that the resulting daughter cells may have the same size of the parental cells at the beginning of G_1. If cell size were not to double between the beginning of G_1 and the end of G_2, cells would become smaller at every division…. and eventually vanish (Fig. 1). Thus, cell size per se does not determine the rate of cell proliferation. One cell line in my laboratory, R-/v-src cells[6] grow very fast even in

serum-free medium, although they are very small. They still have to double in size before each cell division.

Growth in size necessary for cell division. In case you are not convinced, there is experimental evidence that cells have to double in size before dividing.[7,8] Cell size increases during the cell cycle. To quote from Fraser and Nurse[9]: "The two daughter cells produced at each division are identical to the parent at the same time in the preceding cycle: this requires that all components are doubled during the course of each cell cycle". This is an important point. It establishes that growth in size is a fundamental growth process, as growth in cell number also depends, at least in part, on growth in size.

THE IGF AXIS AND CELL GROWTH

The evidence for a role of the insulin-like growth factor (IGF) axis in the regulation of cell size and body size is substantial. Interestingly, all experiments agree that ablation of IGF-I receptor (IGF-IR) signaling does not lead to cessation of normal growth. Rather, it leads to a decrease in body size, as if the IGF axis were responsible for about 50–60% of normal growth in vivo, but no more. The conclusions are two: (1) there are other pathways for animal growth, but (2) IGF-IR signaling is an important component, and a fraction of growth cannot be replaced by other mechanisms. Efstratiadis and collaborators have established the rules on the role of the IGF axis in mouse embryo growth.[10] The IGF-II/IGF-IR interaction accounts for 66% of mouse embryo growth. When only the IGF-IR is deleted, the mice at birth are 46% in size, suggesting that another receptor may partially substitute for the IGF-IR. Subsequent experiments, in vivo and in vitro, have shown that the residual growth occurring in the absence of the IGF-IR, but in the presence of IGF-II, is due to the activation of the insulin receptor (InR) by IGF-II,[11] specifically the A isoform of the InR.[12] The IGF-II receptor down-regulates IGF-II, and its deletion actually results in mice at birth 150% in size in respect to wild type littermates. The role of IGF-IR signaling in determining cell and body size in mouse embryo is supported by other findings. The insulin receptor substrate-1 (IRS-1) is a docking protein for both the IGF-IR and the InR that transmits their signals through PI3-K, Akt and S6K to the nuclei (see below). In *Drosophila* all these homologues have been reported to regulate cell size and body growth.[13-15] Particularly instructive is the *Drosophila* IRS homologue, called chico. Deletion of chico reduces fly weight by 65% in females and 55% in males. The reduction in body and organ size is due both to a reduction in cell number and cell size.[14] Mice with a targeted disruption of IRS-1[16] or S6K1[17] genes are smaller than their wild type littermates, thus confirming the *Drosophila* data. For other genes regulating mouse embryonic growth, see the review by Efstratiadis,[10] who pointed out that the IGF-IR is the only growth factor receptor, so far, whose knock-out has a growth phenotype. Again, I would like to reiterate that the IGF axis is responsible for part of the growth (roughly 50%), but not all of it.

THE INSULIN RECEPTOR SUBSTRATE-1 AND THE INSULIN RECEPTOR PARADOX

The IRS proteins have in common an amino-terminal that includes a Pleckstrin homology (PH) domain and a phosphotyrosine binding (PTB) binding domain. These two domains occupy roughly 300 amino acids, and are followed by long tails, which vary in the various IRS proteins, and contains a number of binding sites for different substrates (PI3-K, Grb2, phosphatases, etc.). IRS-1 is translocated to the nuclei of cells expressing the human or simian

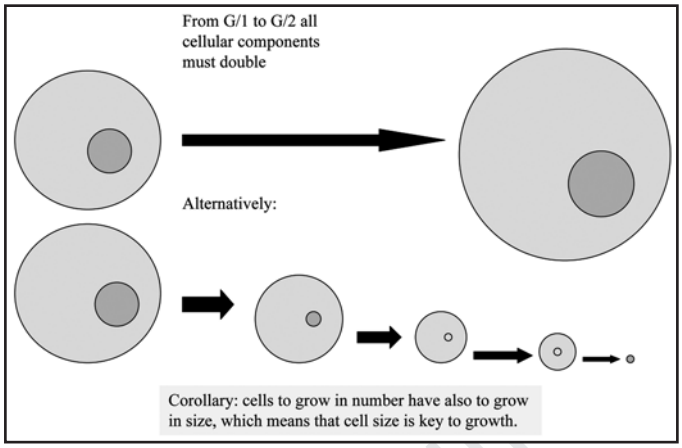

Figure 1. Cells to grown in number have also to grown in size, which means that cell size is key to growth.

viral T antigens,[10,18] and of cells stimulated with IGF-1.[20,21] Nuclear localization of IRS-1 is also found in tissue sections of human breast cancers,[22,23] human medulloblastoma[18] and adult rat liver.[24] In the nucleus, IRS-1 binds the Upstream Binding Factor I (UBF1), one of the proteins that regulate the activity of RNA polymerase I, and therefore cell size (see above). This was an interesting finding, as it opened new vistas on how IRS-1 may regulate cell proliferation, cell growth in size and differentiation. It also provided a molecular explanation for the effect of IRS genes deletion on the cell and body size of flies and mice (see above). IRS-1 is found in nuclei of 32D IGF-IR/IRS-1 cells growing exponentially. When these cells are induced to differentiate along the granulocytic pathway by rapamycin, IRS-1 is exclusively localized to the cytoplasm.[25] Therefore, in most cells of the animal body, that are well differentiated, one would expect to find a cytosolic IRS-1. We stated above that IRS-1 is also the docking protein of the InR, and it is therefore seemingly paradoxical that deletion of the InR genes results in mice that are normal in size at birth.[26]

EXPLANATION OF THE PARADOX

To explain the InR/IRS-1 paradox, we turned to 32D myeloid cells. These are murine myeloid precursor cells that behave in culture like myeloid precursor cells behave in the bone marrow, i.e., they can proliferate but they can also be induced to differentiate by appropriate growth factors, like granulocytic colony-stimulating factor (G-CSF) or IGF-1. Parental 32D cells grow exponentially in Interleukin-3 (IL-3) and do not express IRS proteins (1 or 2). They also express very low levels of IGF-IR. If the IGF-IR levels are increased to normal, cells differentiate when IGF-I replaces IL-3 in the cultures.[27] Ectopic expression of IRS-1 in these cells inhibits differentiation, the cells become transformed and they actually form tumors in mice.[6] IRS-1 also causes these cells to double in size when they are stimulated with IGF-1.[6,21] Interestingly, the combination InR/IRS-1 fails to double the size of the cell,[21] which explains why deletion of the InR genes in mouse embryos does not give a decreased body size. This is probably due to the fact that IGF-1 stimulation is much more effective than insulin stimulation in inducing translocation of IRS-1 to the nuclei.[12,20] It is true that the InR compensates in part when IRS-2 is expressed in 32D cells instead of IRS-1, but it still is not as effective as the combination IGF-IR/IRS1.[21]

CONCLUSIONS

There are of course many ways to regulate cell and body size. After all, deletion of the IGF-IR or IRS-1 genes produces mouse embryos that are 50% in size. It means that IGF-IR/IRS-1 signaling controls in a non-redundant way 50% of mouse embryo size, but it also means that 50% of the size is under the control of other growth factors and/or other agents. Interestingly, deletion of other growth factor receptors in mouse embryos does not give a growth phenotype. It may give a lethal phenotype, as in the case of the PDGF receptor, but not a neat growth phenotype as deletion of the IGF-IR genes gives.[10] Since IRS-1 binds to UBF1 and since it is well known that IGF-1 causes an increase in rRNA synthesis (see for instance Tu et al., ref. 20), it is hardly surprising that deletion of the IGF-IR or IRS-1 genes results in smaller size. Is this important for cell proliferation? When IRS-1 is down-regulated, cells have a tendency to differentiate.[28,29] Differentiation usually affects cell proliferation, although the two are not necessarily incompatible. Regardless of its effect on cell proliferation, IRS-1 signaling clearly plays a role in cell and body size. The next question is the relationship of IRS-1 to the other signal transducing molecules that are known to play a major role in cell size: PI3-K and mTOR.[30] The next wave of experiments should dissect the complicated relationships between these three proteins.

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