Growth factors regulate cell survival by controlling nutrient transporter expression

A.L. Edinger1

Abramson Family Cancer Research Institute, University of Pennsylvania, 450 BRB II/III, 421 Curie Blvd., Philadelphia, PA 19104, U.S.A.

Abstract
Growth factors provide permission signals that allow mammalian cells to grow, proliferate and survive. One mechanism by which growth factors maintain this control is through the regulation of cell surface nutrient transporter expression. Following growth factor withdrawal, nutrient transporters are endocytosed and degraded in the lysosome, effectively terminating the cell’s ability to obtain nutrients. This results in a state of pseudostarvation in which cells atrophy and initiate a catabolic metabolic programme in the midst of abundant extracellular nutrients. Oncogenic forms of Akt can support growth factor-independent nutrient transporter expression through a mechanism that depends upon mTOR (mammalian target of rapamycin). The ability of activated Akt to support nutrient transporter expression is an essential component of its prosurvival function. When the destruction of nutrient transporters is inhibited, cells are capable of long-term growth-factor-independent cell survival in the absence of receptor-dependent signal transduction. These results imply that proteins involved in nutrient transporter turnover in response to growth factor withdrawal are components of a novel tumour suppressor pathway. Preliminary data suggest that Rab7, a GTPase required for transporter degradation, functions as a tumour suppressor protein, as inhibiting Rab7 activity promotes colony formation in soft agar. These studies indicate that drugs affecting this pathway might have utility as anti-cancer chemotherapeutic agents.

Key words: Akt, apoptosis, growth factors, mTOR, Rab7, rapamycin.

Abbreviations used: LDL, low-density lipoprotein; mTOR, mammalian target of rapamycin.

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growth factors and Akt also regulate the energy state of the mitochondria. Following growth factor withdrawal, the mitochondrial membrane potential declines. Expression of myristoylated Akt can completely prevent this change [6,11]. However, the ability of Akt to support growth factor-independent cell survival is completely dependent on the availability of extracellular glucose. While activated Akt protects from growth factor withdrawal-induced death, myristoylated Akt-expressing cells are as sensitive as control cells to glucose withdrawal [11].

The dependence of cells on growth factors and Akt for glucose uptake and metabolism raised the possibility that access to other extracellular nutrients was also regulated. When the localization of the amino acid transporter associated protein 4F2hc was examined by immunofluorescence, it was discovered that 4F2hc is actively removed from the cell surface and degraded in lysosomes following growth factor withdrawal [6]. Thus, in addition to interrupting the transcription and translation of new amino acid transporter proteins, growth factor withdrawal results in the active destruction of those proteins currently expressed on the cell surface.

Actively growing cells require high levels of cholesterol to synthesize new cell membranes. Cells acquire extracellular cholesterol in the form of LDL (low-density lipoprotein) particles. Like glucose and amino acid transporter expression, LDL receptor surface expression is also regulated by growth factors and Akt [6]. Iron, an important component of cellular enzymes such as cytochrome oxidase and ribonucleotide reductase, is taken up from the extracellular space bound to transferrin. Expression of the transferrin receptor is similarly dependent upon the availability of growth factors and activation of Akt [6]. Thus, growth factors and Akt regulate not only the ability to take up glucose from the extracellular space, but also the expression of transporters for a wide variety of extracellular nutrients (Figure 1). When growth factors are withdrawn, these transporter proteins are no longer synthesized and existing transporters are rapidly removed from the cell surface and degraded, resulting in compromised cellular bioenergetics and loss of mitochondrial homeostasis, culminating in the release of cytochrome c and the initiation of apoptosis (Figure 2).

Growth factors and Akt control nutrient transporter expression. In order to test whether this function plays a central role in regulating cell survival, myristoylated Akt-expressing cells were withdrawn from growth factor and treated with the specific mTOR (mammalian target of rapamycin) inhibitor, rapamycin [6]. In yeast, it has been clearly shown that the TOR kinase regulates the expression of a variety of nutrient transporter genes [12]. mTOR appears to lie downstream of Akt in the growth factor signal transduction cascade. Therefore, if Akt exerts its effects on nutrient transporter expression through mTOR, rapamycin treatment will inhibit this function of Akt and the effect on Akt-mediated survival can be measured. Treatment of cells with rapamycin blocked the growth factor-independent expression of nutrient transporters supported by activated Akt. Consequently, rapamycin interfered with the ability
of Akt to maintain mitochondrial membrane potential in the face of growth factor withdrawal and diminished the growth factor-independent cell survival seen in cells expressing oncogenic forms of Akt. These results are particularly intriguing in light of the fact that rapamycin analogues are currently in clinical trials as cancer chemotherapeutics [13]. Most studies examining the mechanism by which rapamycin treatment inhibits tumour cell growth are focused on the role of mTOR in regulating translation [14]. The studies summarized here suggest that rapamycin may also have a negative impact on tumour cell growth by interfering with nutrient transporter expression and that this alternative mechanism should also be examined.

Although growth factors and Akt regulate the expression of nutrient transporters and this is an important component of their prosurvival function, it was not clear that maintaining nutrient transporter expression in the absence of growth factors would be sufficient to maintain cell survival. In order to address this point, growth factor-independent nutrient transporter expression was maintained in the absence of signal transduction by interfering with the function of Rab7. Rab7 is a small, Ras-related GTPase whose function is restricted to modulating fusion events between late endosomes and lysosomes [15]. When Rab7 is inhibited, proteins that have entered the endocytic pathway and would normally be trafficked to the lysosome and degraded are instead shunted into a recycling pathway and re-expressed on the cell surface (Figure 3).

Rab7 function was blocked by expressing the dominant-negative mutant T22N or by expressing short DNA hairpins directed against Rab7 to decrease Rab7 protein levels through the RNAi (RNA interference) pathway. In cells in which Rab7 function was blocked, nutrient transporter expression was maintained despite growth factor withdrawal [16]. In addition, when Rab7 function was blocked, mitochondrial membrane potential was maintained in the absence of growth factor signal transduction. Consistent with this observation, interfering with Rab7 promoted long-term growth factor-independent cell survival. Thus, Rab7 is required for the down-regulation of nutrient transporter proteins following growth factor withdrawal (Figure 2). By blocking Rab7 activity, growth factor-independent nutrient transporter expression can be maintained, promoting nutrient uptake and mitochondrial homeostasis despite growth factor withdrawal (Figure 3).

Based on these results, Rab7 might function as a tumour suppressor gene. As a preliminary test of this idea, primary mouse embryonic fibroblasts that were genetically null for p53 and in which Rb function was inhibited by expression of the adenoviral E1A protein were evaluated for the ability to form colonies in soft agar in the presence or absence of dominant-negative Rab7. Loss of the tumour suppressor genes p53 and Rb was not sufficient to promote colony formation in soft agar. However, providing a mechanism for cell autonomous nutrient uptake in these cells by blocking Rab7 function did promote colony formation [16]. Thus, Rab7 may represent an entirely new class of tumour suppressor proteins that function by controlling nutrient uptake at the level of nutrient transporter expression. Furthermore, chemical agents that affect this pathway may inhibit tumour cell growth and could be useful as cancer chemotherapeutics by controlling nutrient import on the cellular level much as angiogenesis inhibitors limit nutrient availability on the tissue level. It will be interesting and important to determine whether rapamycin works as a cancer chemotherapeutic in part by affecting nutrient transporter expression.

References