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Review

Anoikis: A necessary death program for anchorage-dependent cells

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ABSTRACT

Cell to matrix adhesion is a key factor for cellular homeostasis and disruption of such interaction has adverse effects on cell survival. It leads to a specific type of apoptosis known as "*anoikis*" in most non-transformed cell types. This kind of apoptosis following loss of cell anchorage is important for development, tissue homeostasis and several diseases. Integrins sense mechanical forces arising from the matrix, thereby converting these stimuli to downstream signals modulating cell viability. Anchorage-independent growth is a crucial step during tumorigenesis and in particular during the metastatic spreading of cancer cells. The disruption of the tight control leading an "homeless" cell to death is therefore able to violate the cell defences against transformation. This review analyses the recent investigations into the molecular mechanisms governing *anoikis*, discussing the different ways in which adhesion can influence this process and addressing the relevance of this unique apoptosis mode in the development of metastatic cancers, as well as in other diseases.

Contents

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Abbreviations: DR5, death receptor 5; ECM, extracellular matrix; EGF, epidermal growth factor; EMT, epithelial to mesenchymal transition; ERK, extracellular signal-regulated kinase; FADD, Fas-associated death domain protein; FAK, focal adhesion-kinase; FGF, fibroblast growth factor; FLIP, FLICE inhibitory protein; HGF, hepatocyte growth factor; JNK, Jun-NH2-terminal kinase; IL, interleukin; ILK, integrin-linked kinase; NF-kB, nuclear factor-kB; OMM, outer mitochondrial membrane; PDGF, platelet-derived growth factor; PI3K, phosphoinositide-3-OH kinase; PKB, protein kinase B; ROS, reactive oxygen species; TNFα, tumor necrosis factor-α; TNFR, TNF-α receptor; TNFR-1, TNF-α receptor 1; TGFβ, transforming growth factor β; TRAIL, TNFR apoptosis-inducing ligand; VDAC, voltage-dependent anion channels; VEGF, vascular endothelial growth factor.

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1. The physiological meaning of *anoikis* and the control from extracellular matrix (ECM)

Anoikis, a Greek word meaning 'homelessness', is apoptosis induced by loss of cell adhesion or inappropriate cell adhesion [1]. Adhesion on the extracellular matrix (ECM) is important to determine whether a cell is in the correct location and to remove displaced cells by apoptosis. *Anoikis* following loss of cell anchorage, is of physiological relevance for development, tissue homeostasis and disease [2,3]. *Anoikis in vivo* may prevent detached cells from reattaching to new matrices and growing dysplastically. Of course this could be an important safeguard for the organism.

Integrins regulate cell viability through their interaction with the extracellular matrix and they can sense mechanical forces arising from the matrix, converting these stimuli to chemical signals capable of modulating intracellular signal transduction [4]. *Anoikis* has been described in several cell types, although it appears that cells of different tissue origin activate dissimilar pathways leading to *anoikis*. The physiological relevance of *anoikis* is confirmed by the fact that cancer cells lines, rather than normal epithelial cells, are usually not sensitive to *anoikis*, and many have developed anchorage independence, meaning they do not require adhesion to ECM to proliferate and survive [5–8]. Therefore, the ability to overcome this requirement has important implications for metastatic cancer. Indeed in neoplastic cells, alterations in cell–cell adhesion molecules, protein kinases or phosphatases, integrin-associated signalling molecules or apoptosis regulators can lead to resistance to the physiologically occurring *anoikis*, conferring by this way a constitutive pro-survival signal allowing dissemination of metastatic cancer cells [7–11].

2. The molecular program of *anoikis*

Apoptosis in response to lack of adhesion or inappropriate adhesion to the ECM has been termed *anoikis*, but, in spite of its unique definition, *anoikis* is essentially an apoptotic process.

The initiation and execution of *anoikis* is mediated by different pathways, all of which merge into the activation of caspases and downstream molecular pathways, culminating in the activation of endonucleases, DNA fragmentation and cell death. In particular, *anoikis* seems to occur following either the perturbation of mitochondria (the intrinsic pathway) or the triggering of cell surface death receptors (the extrinsic pathway) (Fig. 1, panel B) [3,12].

The proteins of the Bcl-2 family are key arbiters of both these processes [7]. The Bcl-2 family can be divided into three groups: (i) the anti-apoptotic proteins, including Bcl-2, Bcl-XL and myeloid cell leukaemia sequence 1 (Mcl-1); (ii) the multidomain pro-apoptotic proteins Bax, Bak and Bok; (iii) the pro-apoptotic BH3-only proteins, counting Bid, Bad, Bim, Bik, Bmf, Noxa, Puma and Hrk.

In the intrinsic pathway, caspase activation occurs as a consequence of mitochondrial permeabilization [13,14]. This pathway is regulated by the pro-apoptotic proteins Bax and Bak, which form oligomers in the outer mitochondrial membrane (OMM), creating channels within this membrane, thus causing its permeabilization. It has been postulated that membrane permeabilization may result not only from the intrinsic pore forming activity of the Bax proteins, but even from their interaction with mitochondrial channel proteins such as the voltage-dependent anion channels (VDACs) [15]. However, recent data obtained by Baines et al. indicate that VDACs are dispensable for Bcl-2 family member-driven cell death, since wild-type and VDAC-deficient cells exhibited equivalent cytochrome *c* release, caspase cleavage and cell death in response to the pro-death Bcl-2 family members Bax and Bid [16]. The consequence is the disruption of the OMM and the release of cytochrome *c*, leading to formation of the so-called apoptosome, composed of caspase-9, the cofactor apoptosis protease activating factor (Apaf) and cytochrome *c*, with subsequent activation of the effector caspase-3 and execution of the apoptotic process [17–19]. This intrinsic cascade is initiated by the pro-apoptotic BH3-only family of proteins, essential players during the *anoikis* programme [20]. In particular, among the members of this family, Bid and Bim are activated following detachment of cells from ECM and rapidly promote the assembly of Bax-Bak oligomers within the OMM. These members of the BH3-only protein family are termed activators [21].

The anti-apoptotic Bcl-2 family of proteins are structurally related to the Bax and BH3-only families but prevents apoptosis by maintaining mitochondrial membrane integrity and avoiding pore formation and OMM disruption, although the precise relationships between these proteins are not yet completely defined [2,21–23]. The original model of Bcl-2 for the inhibition of Bax function through heterodimerization is no longer valid because of the difficulty in detecting this complex under physiological conditions. Additionally, Bcl-2 family proteins can inhibit apoptosis by sequestering the activator members of the BH3-only proteins, namely Bid and Bim, thereby preventing oligomerization of Bax and Bak [24,25]. There is another group of the BH3-only proteins, called sensitizers, which are unable to directly activate Bax and Bak oligomerization [25,26]. The sensitizer BH3-only proteins (Bad, Bik, Bmf, Noxa, Puma and Hrk) contribute to cell death by competing for the Bcl-2's BH3 binding domain, inactivating the anti-apoptotic functions of Bcl-2, thus freeing activator BH3-only proteins to induce Bax-Bak oligomer formation [27,28].

Several evidences indicate that the BH3-only proteins play a role in *anoikis* execution of different cell types. Noxa and Puma are transcriptionally regulated by p53 and have been implicated in fibroblast *anoikis* [29,30]. Bim and Bad can be controlled by the phosphoinositide-3-OH kinase (PI3K) and extracellular signal-regulated kinase (ERK) pathways, and Bid may be cleaved in the death receptor pathway [31–34]. In particular, Bim is sequestered in the dynein complex until the loss of integrin engagement induces its release and translocation to mitochondria, where it interacts with Bcl-XL neutralizing its pro-survival function [35]. In addition, both ERK and PI3K/Akt-mediated phosphorylation of Bim, elicited upon integrin engagement, leads to the proteasome-dependent degradation of Bim [36,37]. As a consequence, the loss of ECM contact, leading to inhibition PI3K/Akt and ERK signals, strongly increases Bim accumulation [35]. The BH3-only protein Bmf has been implicated in *anoikis* through its interaction with the myosin V motor complex, although further evidence is required to show whether it is activated in