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Review

Anoikis molecular pathways and its role in cancer progression ☆

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ABSTRACT

Anoikis is a programmed cell death induced upon cell detachment from extracellular matrix, behaving as a critical mechanism in preventing adherent-independent cell growth and attachment to an inappropriate matrix, thus avoiding colonizing of distant organs. As anchorage-independent growth and epithelial–mesenchymal transition, two features associated with *anoikis* resistance, are vital steps during cancer progression and metastatic colonization, the ability of cancer cells to resist *anoikis* has now attracted main attention from the scientific community. Cancer cells develop *anoikis* resistance due to several mechanisms, including change in integrins' repertoire allowing them to grow in different niches, activation of a plethora of inside-out pro-survival signals as over-activation of receptors due to sustained autocrine loops, oncogene activation, growth factor receptor overexpression, or mutation/upregulation of key enzymes involved in integrin or growth factor receptor signaling. In addition, tumor microenvironment has also been acknowledged to contribute to *anoikis* resistance of bystander cancer cells, by modulating matrix stiffness, enhancing oxidative stress, producing pro-survival soluble factors, triggering epithelial–mesenchymal transition and self-renewal ability, as well as leading to metabolic deregulations of cancer cells. All these events help cancer cells to inhibit the apoptosis machinery and sustain pro-survival signals after detachment, counteracting *anoikis* and constituting promising targets for anti-metastatic pharmacological therapy. This article is part of a Special Section entitled: Cell Death Pathways. Guest Editors: Frank Madeo and Slaven Stekovic.

1. Introduction

In the absence of attachment to extracellular matrix (ECM) or upon cell adhesion to inappropriate location, cells undergo a particular type of apoptosis, termed *anoikis*, a Greek word meaning loss of "home" or "homelessness". Indeed, integrin receptors, as mediators of cell–ECM interaction, not only provide physical links with the cytoskeleton but also transduce signals from the ECM to the cell, mandatory for several cellular processes including migration, proliferation and survival [24,71,72,84,180]. *Anoikis*, first described in epithelial and endothelial cells [71], can be viewed as a physiologically relevant process which ensures development and tissue homeostasis. *Anoikis* acts as an important defense for the organism by preventing detached cells' re-adhesion to new matrices in incorrect locations and their dysplastic growth. As a result, failure to execute the *anoikis* program could result in adherent cells surviving under suspension conditions or proliferating at ectopic sites where the ECM proteins are different from the original ones. This deregulation in *anoikis* execution is emerging as a hallmark of cancer cells and contributes to the formation of metastasis in distant organs [47,73,92,217].

2. Molecular pathways of *anoikis*

The initiation and execution of *anoikis* is mediated by different pathways, all of which terminally converge into the activation of caspases and downstream molecular pathways, culminating in the activation of endonucleases, DNA fragmentation and cell death. The induction of the *anoikis* program occurs through the interplay of two apoptotic pathways, namely the perturbation of mitochondria (the intrinsic pathway) or the triggering of cell surface death receptors (the extrinsic pathway) [92,98] (Fig. 1). The proteins of the Bcl-2 family are key players of both

Abbreviations: AMPK, AMP activated protein kinase; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; EMT, epithelial–mesenchymal transition; ERK, extracellular signal-regulated kinase; FLIP, FLICE inhibitory protein; FAK, focal adhesion kinase; GSK-3, glycogen synthase kinase-3; HGF, hepatocyte growth factor; HIF, hypoxia-inducible factor; IL, interleukin; ILK, integrin-linked kinase; IKK, IκB kinase; MAPK, mitogen activated protein kinase; Mcl-1, myeloid cell leukemia sequence 1; MET, mesenchymal epithelial transition; MMP, metalloproteinase; NFκB, nuclear factor-κB; Nox, NADPH oxidase; OMM, outer mitochondrial membrane; PDGFR, platelet-derived growth factor receptor; PDK, pyruvate dehydrogenase kinase; PERK, protein kinase like endoplasmic reticulum kinase; PI3K, phosphoinositide-3-OH kinase; PIP3, phosphatidylinositol 3,4,5-triphosphate; PKB, protein kinase B; PK-M2, pyruvate kinase isoform-2; PPP, pentose phosphate pathway; PTEN, phosphatase and tensin homolog; ROS, reactive oxygen species; RTKs, receptor tyrosine kinases; SMA, α-smooth muscle actin; TNFR, tumor necrosis factor receptor; TrkB, tyrosine kinase receptor B; VEGFR, vascular endothelial growth factor receptor

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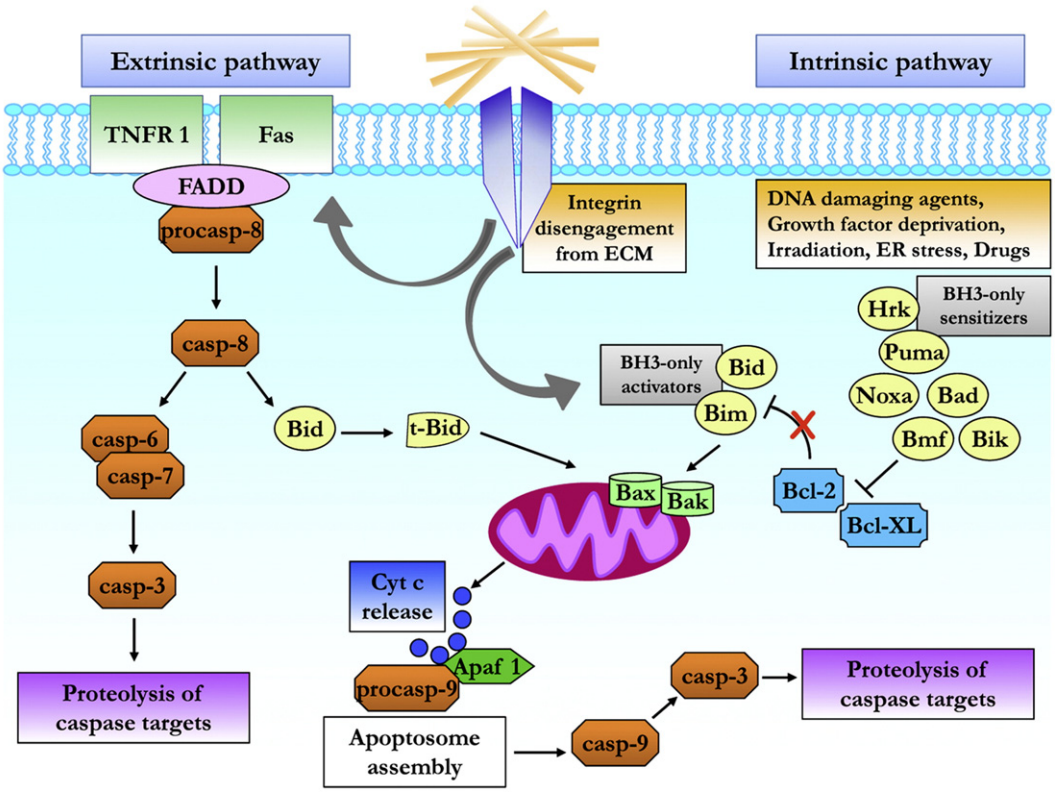

Fig. 1. Extrinsic and intrinsic apoptotic pathways. The lack of ECM contact or the engagement with inappropriate ECM leads to the activation of *anoikis* from death receptors (extrinsic pathway) and mitochondria (intrinsic pathway). In the extrinsic pathway of apoptosis, caspase-8 is activated upon engagement of death receptors (i.e., Fas or TNFR1), leading to cleavage and activation of executioner caspases (for example, caspase-3). In the intrinsic pathway, Bax/Bak activation is promoted by BH3-only proteins, such as Bim, Bad, Bik, Puma, Hrk, Bmf and Noxa. Among them, Bid and Bim (activators), directly promote the assembly of Bax–Bak oligomers, while the others BH3-only members (sensitizers), counteract the anti-apoptotic functions of Bcl-2, thus indirectly inducing Bax/Bak activation. As a final outcome, cytochrome c is released to the cytoplasm, where it induces the formation of the apoptosome, leading to activation of executioner caspases.

these processes. The Bcl-2 family can be divided into three groups: (i) the anti-apoptotic proteins, including Bcl-2, Bcl-XL and myeloid cell leukemia sequence 1 (Mcl-1); (ii) the multidomain pro-apoptotic proteins Bax, Bak and Bok; and (iii) the pro-apoptotic BH3-only proteins, counting Bid, Bad, Bim, Bik, Bmf, Noxa, Puma and Hrk [180].

2.1. The intrinsic pathway

The intrinsic pathway is triggered in response to several intracellular signals, including DNA damage and endoplasmic reticulum stress, where mitochondria play a central role with regard to the control of apoptosis [132]. In response to death signals, the pro-apoptotic protein Bax and Bak translocate from the cytosol to the outer mitochondrial membrane (OMM), where their oligomerization creates a channel within the OMM, causing mitochondrial permeabilization and cytochrome c release. In addition to the intrinsic pore forming activity of the Bax proteins, membrane permeabilization may result even from their interaction with mitochondrial channel proteins such as the voltage-dependent anion channels [206]. The release of cytochrome c leads to the 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 [48,224,266].

The pro-apoptotic BH3-only proteins act as critical players during the intrinsic cascade of the *anoikis* program [25]. 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 OMM. These members of the BH3-only protein family are termed "activators" [221]. In particular, Bim is sequestered in the dynein cytoskeletal complexes until cell detachment induces release of Bim from these structures and causes its translocation to the mitochondria [45]. Loss of cell adhesion also causes Bim accumulation, through the inhibiting of its proteasomal degradation initiated by an extracellular signal-regulated kinase (ERK) and phosphoinositide-3-OH kinase (PI3K)/Akt-mediated phosphorylation of Bim, elicited upon integrin engagement [45,138,176].

Another group of the BH3-only proteins are termed "sensitizers" and includes Bad, Bik, Bmf, Noxa, Puma and Hrk. The sensitizer BH3-only proteins are unable to directly activate Bax and Bak oligomerization and contribute to cell death through the inactivation of the anti-apoptotic functions of Bcl-2, by competing for its BH3 binding domain, thus freeing activator BH3-only proteins to induce Bax–Bak oligomer formation [14,135,140,231]. Indeed, Bcl-2 is the master anti-apoptotic member of the family which avoids mitochondrial dysfunction and prevents apoptosis both by interacting with Bak/Bax apoptotic members, thus avoiding their clustering into pores and by sequestering the activator members of the BH3-only proteins, namely Bid and Bim, thereby preventing Bak/Bax oligomerization [79,231].

Compelling evidence indicate the involvement of other members of the BH3-only family in *anoikis* execution of different cell histotypes. For example, Noxa and Puma are transcriptionally regulated by p53 and have been implicated in fibroblast *anoikis* [162,203]. Furthermore, in epithelial cells the Bcl-2 modifying factor (Bmf) behaves as a sentinel able to register damage at the cytoskeleton and to convey death signals. Indeed, upon cell detachment, Bmf is released from its previous interaction with the myosin V motor complex [175] and accumulates in the mitochondria, where it neutralizes Bcl-2, leading to cytochrome c release and *anoikis* execution [203].

2.2. The extrinsic pathway

Alongside the intrinsic pathway, extrinsic pathway also contributes to *anoikis* execution. The extrinsic pathway is initiated by ligand binding of members of the tumor necrosis factor receptor (TNFR) superfamily of death receptors, such as Fas receptor, TNFR1 and the TNF-related apoptosis inducing ligand (TRAIL) receptor-1 and -2, resulting in the formation of the death-inducing signaling complex (DISC). In turn, DISC, via the interaction with adaptor proteins such as the Fas-associated death domain protein (FADD), recruits several molecules of caspase-8, thereby promoting their activation. Active caspase-8 is then released to the cytoplasm where it cleaves and activates the effectors caspase-3, -6, and -7, culminating in substrate proteolysis and cell death [221,235]. Alternatively, caspase 8 activation results in the cleavage and activation of Bid, which in its truncated form (t-Bid), can promote mitochondrial cytochrome c release and assembly of the apoptosome, thus linking the extrinsic pathway to the intrinsic one [230]. Recent evidence reported that upon cell detachment from ECM, a mitochondrial protein, named Bit1, is released to the cytoplasm and acts as a pro-apoptotic mediator, inducing a caspase-independent form of apoptosis [117,119]. In some instances, activation of the death receptor pathway could be secondary to mitochondrial damage, establishing once again a crosstalk between extrinsic death signals and the intrinsic pathway [188]. Previous studies demonstrated that the loss of anchorage to ECM leads to the upregulation of Fas and Fas ligand and a concomitant downregulation of FLICE inhibitory protein (FLIP), an endogenous inhibitor of Fas-mediated signaling, indicating the important role of the extrinsic pathway to the *anoikis* process [4,183]. Interestingly, also changes in cell shape can induce extrinsic *anoikis* [41]. Indeed, cell rounding following detachment could lead to "induced proximity" of Fas receptors resulting in their activation [161].

As described above, both extrinsic and intrinsic apoptotic pathways merge at and rely on the activation of the effector caspase-3, which initiates a downstream proteolytic cascade to effect cell death. In particular, cleavage of signaling molecules like focal adhesion kinase (FAK) and p130Cas is important for the apoptotic execution [51,180,204,242]. The caspase-mediated cleavage of FAK disrupts focal adhesion architecture and inhibits its survival signal [1]. p130Cas, an SH2/SH3 adaptor protein, which binds FAK and transmits integrin signals, undergoes caspase-mediated cleavage, thus impairing its subcellular localization as well as its interaction with paxillin [204]. Moreover, p130Cas cleavage generates a C-terminal inhibitory fragment which impairs the transcription of p21Waf1/Cip1, thus contributing to the apoptotic response by blocking the cell cycle [130]. Disruption of the cytoskeleton, which accompanies cell–matrix dissociation, may also contribute to *anoikis* induction by releasing pro-apoptotic factors such as Bim [174] or death receptors such as Fas [232] from a sequestered state.

3. Protection from *anoikis* in physiological conditions

3.1. Cell adhesion to ECM

Epithelial cells are protected by *anoikis* when they are adherent on permissive ECM proteins. An enormous amount of literature establishes the central role of integrins in suppressing apoptosis in attached cells by eliciting anti-apoptotic and pro-survival signals from the ECM [72,85]. Integrins are categorized depending on their α or β subunit composition. There at least four types of integrins (α5β1, αvβ3, α1β1 and α6β1) acknowledged to play a role in cell survival [29,154,163,261] in various cellular models. These specific integrins have different abilities to protect cells from apoptosis and *anoikis*, suggesting that they utilize diverse signaling pathways. Their downstream pathways or molecules are different and include FAK [75], Src kinase [169], integrin-linked kinase (ILK) [22], PI3K/Akt [127] and mitogen activated protein kinase (MAPK) [49] (Fig. 2). Activation or overexpression of these signaling molecules has been shown to interfere with *anoikis*, and it is therefore not surprising that some of these molecules have been found to be upregulated or activated in malignant cells.

FAK is one of the most important integrin signaling molecules recruited into focal adhesions upon cell–ECM contact, which affects multiple critical cellular processes such as cell survival, proliferation, motility, and differentiation. FAK is rapidly phosphorylated and activated following integrin-mediated adhesion. The autophosphorylation on Tyr397 recruits and activates Src, which in turn further phosphorylates FAK in the activation loop, yielding the fully active FAK enzyme [36,144]. The activated FAK enables the recruitment of other scaffold and signaling molecules to the focal adhesion sites, consequently activating the downstream cell survival signaling.

PI3K is one of the FAK-activated proteins, which in turn recruits and activates its downstream target protein kinase B (PKB/Akt) [42,247]. Akt activation promotes cell survival by several independent mechanisms. For example, it prevents the release of cytochrome c from mitochondria by phosphorylating the pro-apoptotic protein Bad on Ser136 [53,54,56], causing the release of Bcl-2 and directly inhibits the caspase cascade by phosphorylating the procaspase-9 [38]. Akt can also activate the nuclear factor κB (NFκB) survival pathway by phosphorylating and inhibiting IκB (the inhibitory subunit of NFκB) through IκB kinase (IKK) [182].

The MAPK pathway is another signaling cascade triggered by FAK activation. Several mechanisms are proposed to account for the link between FAK and MAPK [190]. One is the Src-mediated phosphorylation of FAK on Tyr925, which creates a binding site for the Grb2 adaptor protein, leading to the activation of the MAPK pathway [192,194]. Another mechanism for MAPK activation is that the FAK/Src complex promotes the tyrosine phosphorylation of Shc, which in turn leads to the recruitment of Grb2 [195]. Furthermore, FAK may be linked to MAPK by inducing phosphorylation of its binding partner p130Cas which in turn recruits Nck adaptor proteins [191].

At least two mechanisms have been proposed for MAPK during the protection against apoptotic cell death. One is the MAPK-dependent regulation of the pro-apoptotic proteins Bim and Bad. Activated MAPK-1/2 phosphorylates Bim at several sites and this phosphorylation targets Bim for ubiquitination and proteasomal degradation [141]. MAPK activation also specifically prevents upregulation of Bim, thus acting both at transcriptional and posttranslational levels [150]. In addition, MAPK induces Bad phosphorylation, leading to inhibition of its pro-apoptotic function [21,54,145,205,233]. The other mechanism by which survival factors exploit MAPK pathway to protect against apoptotic cell death is the upregulation of the pro-survival members of the Bcl-2 family, such as Bcl-2, Bcl-X_L and Mcl-1, via phosphorylation of the transcription factor cAMP responsing element binding protein (CREB) [193].

ILK is another key player in integrin-mediated signal transduction leading to *anoikis* protection. Upon cell–ECM adhesion, ILK interacts with integrin receptors and phosphorylates its target PKB/Akt on Ser473, thereby stimulating its activity [5,244]. Overexpression of active FAK or ILK blocks *anoikis* in suspended cells, thereby supporting a role of these kinases in *anoikis* protection [71,74,113,114]. Of note, ILK conveys integrin-mediated survival signals independently of FAK, as indicated by the inability of dominant-negative FAK to revert the ILK-mediated protection from *anoikis*, suggesting that the two kinases affect *anoikis* in different and parallel pathways [5,170].

Finally, another integrin-mediated survival signaling occurs through the caveolin-1-mediated binding of integrins to the adaptor protein Shc, which leads to a FAK-independent activation of MAPK and consequently to escape from *anoikis*, as well as cell cycle progression through cyclin D1 accumulation [9,238].

Integrin engagement, besides activating pro-survival pathways dependent on the recruitment of a platform of adaptor proteins and kinases, can result in ligand-independent activation of many growth

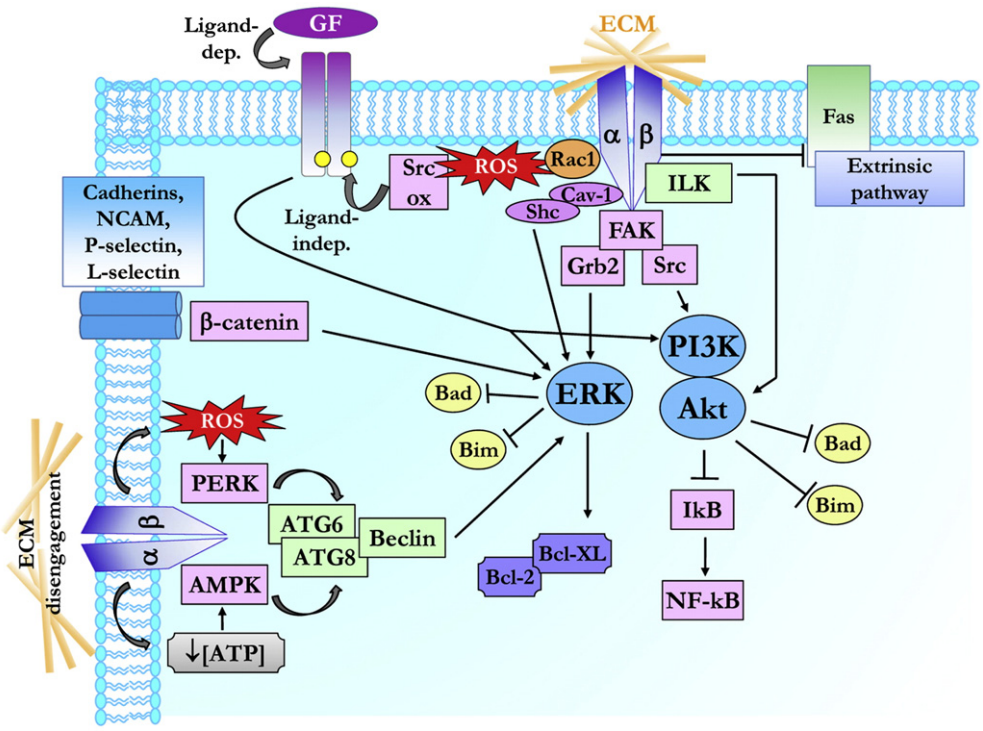

Fig. 2. The molecular signature of cell survival in physiological conditions. Cell adhesion to ECM triggers several pro-survival pathways through the activation of key players (FAK, ILK, Src, Shc), converging on master regulators of *anoikis* resistance, namely PI3K/Akt and ERK. These pro-survival routes promote on one hand the expression and/or activation of anti-apoptotic proteins (Bcl-2, Bcl-XL, NF-κB) and, on the other, the inhibition of pro-survival members (Bad, Bim), thereby preventing the intrinsic pathways of cell death. Integrin engagement also suppresses the expression of Fas, thus interfering with the activation of the extrinsic machinery. Growth factor receptors, activated both in a ligand dependent or independent manner, collaborate with integrin in promoting cell survival. Intercellular adhesion mediated by cadherins or other cell surface molecules, activates signaling pathway similar to those triggered by ECM-adhesion. As a consequence of metabolic and oxidative stress induced by ECM disengagement, an autophagic response sustained by the ATG proteins may provide a temporary survival mechanism, giving cells the chance to survive and reattach to the matrix (see text for details).

factor receptors, such as epidermal growth factor receptor (EGFR) [159], insulin receptor [197], platelet-derived growth factor receptor (PDGFR) [216], receptor for hepatocyte growth factor (HGF), also named Met [236] and vascular endothelial growth factor receptor (VEGFR) [209].

In particular, the ligand-independent phosphorylation of EGFR in response to integrin ligation is strictly dependent by its association with the adaptor protein p130Cas and the Src kinase [158,159]. It has been reported that reactive oxygen species (ROS), produced through the involvement of the small GTPase Rac-1 upon integrin engagement, are responsible for the redox-mediated activation of Src, leading to the ligand-independent *trans*-phosphorylation of EGFR. In turn, redox-activated EGFR switches on both MAPK and PKB/Akt pathways. Both these pro-survival signaling mediators, lead to the phosphorylation and ubiquitin-mediated degradation of Bim, thereby preventing the *anoikis* execution [89]. Conversely, in suspended cells the disengagement of β1 integrin inhibits the expression of EGFR and induces Bim accumulation [202]. In addition, prolonged cell suspension further reduces EGFR expression, thus sustaining the suppression of survival signals, while the re-establishment of integrin-mediated adhesion rescues the levels of EGFR and its pro-survival spur [158].

Besides the above described signaling events initiated upon cell–ECM attachment and leading to cell survival through the suppression of the intrinsic pathway, the extrinsic pathway has also been shown to be inhibited by ECM engagement. Matrix attachment protects endothelial cells from the death receptor Fas-induced apoptosis by suppressing the expression of Fas and an endogenous antagonist of caspase-8, c-FLIP. Regulation of the c-FLIP expression involves MAPK activation in an adhesion-dependent manner, although FAK does not appear to be involved [4].

3.2. Lack of adhesion during cell migration

The second physiological process in which cells need to escape from *anoikis* is the temporary displacement of focal contacts during cell migration. One of the motility style that cells use to migrate is the mesenchymal motility, characterized by an elongated cell morphology with established cell-polarity and dependent upon ECM proteolysis and focal contacts [68,248]. Integrin engagement within focal contacts and the concomitant activation of several receptor tyrosine kinases (RTKs), including Met, which is often the initiating event for mesenchymal motility, leads to PI3K activation and grants for the commitment of a pro-survival signaling [12,248]. In addition, this leads to the PI3K-dependent activation of Rac-1 and Cdc42 at the leading edge of the cell, which coordinate actin polymerization [187]. Mesenchymal motility is clearly linked to pro-survival signals, as several recent reports showed for cells undergoing epithelial–mesenchymal transition (EMT) (see Section 4.3).

The alternative motility style is the amoeboid migration, which allows cells to glide through, rather than degrade, ECM barriers through a weakening of focal contacts. Intriguingly cell–ECM attachments are not required for amoeboid movement and focal adhesions are not organized [68,248]. It is likely that during amoeboid motility, the pro-survival signals are ensured by the strong activation of the Rho family of GTPases. In keeping with this hypothesis, RhoG has been reported to regulate the suppression of *anoikis* in a PI3K-independent manner [249].

The amoeboid movement is also exploited by non-professional adhering cells, among which hematopoietic stem cells and leukocytes [70]. T lymphocytes and other leukocytes move in a protease-independent manner across matrix barriers through adaptation of the

cell shape and squeezing through narrow spaces [243]. The movement of these cells is driven by weak interactions with ECM, thereby permitting high velocities. Since in hematopoietic stem cells and leukocytes integrin-mediated focal adhesions are dispensable, they are unable to ensure pro-survival signals. Hence, it is conceivable that these non-adhering cells are protected from *anoikis* by the anti-apoptotic signal elicited by several cytokines, including interleukin-2 (IL-2), IL-7, IL-15 and interferon-α, which selectively abrogates induction of the pro-apoptotic BH3-only proteins [67]. In keeping with this hypothesis, in quiescent T-cells the withdrawal of survival factors leads to Bim accumulation and Bcl-XL downregulation and final commitment to apoptosis [186].

3.3. Cell–cell contacts

Increasing evidence show that not only cell–matrix adhesion but also cell–cell adhesion supports cell survival. Cell–cell contacts are mainly mediated by cadherins, a family of membrane proteins allowing homotypic or heterotypic calcium-dependent cell–cell anchorage. Cadherins play a crucial role in the complex network of survival signaling. Indeed, it has been reported that blockage of E-cadherin binding induces *anoikis* [17,123], while overexpression of β-catenin, a downstream regulator of cadherin signaling, elicits *anoikis* resistance in epithelial cells [165]. N-cadherin signaling mainly promotes survival in a PI3K/Akt-dependent fashion (see also Section 4.3) [17]. In addition, cadherins may also affect cell survival through indirect association with integrins. Indeed, some integrins, i.e. α2β1 and α3β, can be localized at cell–cell contacts and can mediate survival signals despite loss of ECM adhesion. Fascinatingly, these integrins are functionally associated with the EGFR, suggesting a functional role of EGFR *trans*-activation as a pro-survival signal [255].

Recent evidence highlight that, in addition to cadherin, intercellular adhesion mediated by other cell surface molecules, as P- and L-selectin and NCAM, play an important role in cell survival via the activation of intracellular signaling molecules, similar to those triggered by ECM-adhesion, such as FAK, Src, PI3K/Akt and MAPK [13,31,103].

3.4. Detachment-induced autophagy

On detachment from the ECM, normal epithelial cells show a substantial downregulation in EGFR expression, which results in the inhibition of the pro-survival PI3K/Akt pathway [181]. Both PI3K and Akt have been found to be crucial for glucose transport and metabolism [63] and, accordingly, detached epithelial cells also show a marked reduction in ATP levels, which is a result of the loss of glucose transport. In addition, the impairment in glucose uptake leads to low levels of glucose-6-phosphate and limits flux through the pentose phosphate pathway (PPP). The reduced PPP flux causes an increase in reactive oxygen species (ROS) levels, thereby further inhibiting ATP generation and contributing to the induction of cell death [34]. In this context, autophagy may provide a temporary survival mechanism, which delay the onset of apoptosis, thus giving cells the chance to survive and reactivate once they reattach to the ECM [76,108]. During autophagy, cells package cellular proteins and organelles within the autophagosome. The vesicles are then catabolized by lysosomes and the degraded products are utilized by the cell in order to create new proteins. Induction of autophagy is driven by the activation of proteins that sense cellular metabolic stress, such as AMP activated protein kinase (AMPK) [121,146]. AMPK activates the canonical autophagic pathway through ATG6 and ATG8, thereby sustaining ATP levels and delaying *anoikis* (Fig. 2) [128]. The functional players of such integration are Beclin-1, an autophagic protein acknowledged to modulate the anti-apoptotic role of Bcl-2 and Bcl-XL [264] and MAPKs [7]. In addition to the activation of autophagy by metabolic stress, it has been found that autophagy can be induced also by ECM detachment-induced oxidative stress. This oxidative stress-mediated induction of autophagy has been found to be controlled by the activity of the RNA activated protein kinase like endoplasmic reticulum kinase (PERK) [7]. In turn PERK phosphorylates and activates eukaryotic translation initiation factor 2α, thus inducing transcription and translation of the ATG proteins required for autophagy and sustaining survival to detachment [7].

4. *Anoikis* resistance in cancer cells

Nevertheless non neoplastic cells undergo *anoikis* in response to ECM detachment, cancer cells rapidly develop several mechanisms to resist *anoikis* and exploit them to progress towards malignancy and spread metastases to distant organs. Cancer cells can achieve resistance to *anoikis* through: i) a specific switch in their integrins, thereby adapting to the metastatic site, ii) undergoing to EMT, iii) exploiting a constitutive activation of pro-survival signaling due to intrinsic or environmental factors, as well as iv) deregulating and adapting their metabolism, mainly through Warburg metabolism or autophagy.

4.1. "Integrins switch"

Many experimental evidences demonstrated that both deregulation of integrins and changes in their expression profile can contribute to cancer cells growth or metastatic dissemination. In fact, by changing the integrin repertoire expression, cancer cells can overcome *anoikis* during both the initial phase of oncogenic transformation and metastatic colonization of other organs or tissues.

Several examples of integrins switch have been reported. In human intestinal carcinoma cells, downregulation of αvβ3 integrin expression protects suspended cells from death, suggesting that this contributes to acquisition of an *anoikis* resistant phenotype [160]. Moreover, results obtained from other studies demonstrated that in melanoma cells αvβ3 integrin has a positive role in induction of *anoikis* resistance. Indeed, it has been observed that integrin αvβ3 is expressed in invasive melanoma but not in benign nevi or normal melanocytes suggesting that αvβ3 expression is essential to promote *anoikis* resistance, cancer cells invasion and metastatization [64,80]. The contribution of αvβ3 integrin in the acquisition of a *anoikis*-resistant/migratory cancer cell phenotype, is also confirmed by analyses of different prostate cancer cell lines. In fact, normal prostate epithelial cells and androgen-sensitive LNCaP prostate cancer cell line did not express αvβ3 integrin which results otherwise expressed on androgen-resistant PC3 cancer cell line [263].

Analysis of integrins expression profile reveals that normal squamous cells express prevalently α2β1, α3β1 and α6β4 integrins; on the contrary, αvβ5 integrin, able to activate intrinsic apoptotic pathway when unligated, is expressed at low levels. Transition from normal cells to hyperproliferative as well as cancerous phenotype, is associated with high expression level of pro-survival αvβ6 integrin. This switch strongly contributes to the acquisition of an *anoikis*-resistant phenotype [118]. Integrin α6 expression is also significantly upregulated in numerous carcinomas, including head and neck cancers and breast cancers [77,155]. In normal cells, integrin β6 is expressed during development from a subset of epithelial cells of kidney, lung, and skin, but became undetectable in the adult normal cells. In contrast, according to physiophatological role in the acquisition of *anoikis* resistance and in the invasion process, high level expression of integrin β6 can be detected in several types of carcinoma cells [32]. Overexpression of the β6 subunit into poorly invasive oral squamous cell carcinoma stimulate migration and secretion of metalloproteinase-3 (MMP-3) that, in turn, stimulate cell invasion [179]. On the other hand, it is well known that MMP expression positively correlates with EMT (see also Section 4.3), suggesting that integrin β6 expression is correlated with *anoikis* resistance. Moreover, other mechanisms link integrins action with *anoikis* resistance. For example, it has been observed that overexpression of β4 integrin

causes a constitutive activation of PI3K, inducing *anoikis* resistance and a strong increase of breast cancer cells invasiveness, while β4 knockdown promotes apoptosis [20].

4.2. Constitutive activation of anti-apoptotic pathways

Detached or migrating cancer cells can adopt different strategies to compensate the loss of integrins signals and overcome *anoikis*. PI3K/Akt is one the most important signaling pathway involved in pro-survival features, as it integrates most of the signals derived from integrins and growth factors receptors. Akt is essential to regulate several cellular functions such as cell survival and cell growth, and its aberrant or constitutive activation strongly contributes to sustain cancer growth [2,40,166,184,198]. Sustained pro-survival Akt activation can be achieved as a consequence of i) overexpression or constitutive activation of several receptor protein tyrosine kinases, ii) activating Ras mutations, iii) loss of the phosphatase and tensin homolog (PTEN) function via gene mutation, deletion, or promoter methylation, iv) alteration of PI3K activity, v) amplification of Akt genes or overexpression. Overall, Akt activation can modulate activity of transcription factors that control the expression of pro- and anti-apoptotic genes or direct phosphorylation of pro-apoptotic proteins, such as Bad and procaspase-9, inhibiting their function. In addition Akt activates the transcription factors that upregulate anti-apoptotic genes such as IKK. Finally, it has been demonstrated that Akt negatively regulates the transcription factors that promote the expression of death genes, such as forkhead transcription factors, FKHR, FKHRL1, and AFX [40,166,184,198,225]. Sustained Akt activation occurs also following upregulation of N-cadherin expression. It is well known that switch from E-cadherin to N-cadherin is a common feature of cancerous epithelial cells that undergo EMT [83]. N-cadherin recruits PI3K which in turn activates Akt and induces *anoikis* resistance [39]. The role of EMT in the *anoikis* resistance acquisition, is described in details in Section 4.3.

Activation of PI3K/Akt signaling pathway is the most common mechanism to achieve *anoikis* resistance in cancer cells and PTEN is its most important negative regulator. Loss of function mutations, downregulation or inhibition of PTEN, a very common feature of cancer cells, is often correlated with achievement of *anoikis* resistance and malignancy [55,234]. On the contrary, overexpression of PTEN triggers *anoikis*, mainly via suppression of both FAK and Akt phosphorylation [55].

Other mandatory elements of *anoikis* signaling pathways are Src family kinases. Based on the present knowledge, it is widely accepted that low levels of Src activity is required to maintain integrity of the epithelium in normal tissue. On the contrary, activation of Src, Fyn or Yes kinases leads to cell–cell contacts disassembling and induces scattering in both normal and tumor-derived epithelial cells through activation of integrins and FAK signaling, while deletion of *fyn* and *src* genes is correlated with the appearance of skin architecture abnormalities [27]. Moreover, elevated Src activity stimulates endocytosis of E-cadherin through activation of the E3 ubiquitin ligase or via the Arf-6 GTPase, favoring a mesenchymal-like phenotype [8]. Overall, these evidence suggest that Src activity enhances EMT, an event commonly correlated with *anoikis* resistance (see Section 4.3). In cancer cells sustained Src activation leads to constitutive phosphorylation of FAK on tyrosine 397, allowing PI3K recruitment. This, in turn, activate Akt that inhibits apoptosis by regulating various components of the cell death machinery, including the pro-apoptotic Bim. Phosphorylated FAK acts as docking site for Grb2, leading to activation of the Ras/MAPK pathway, that in turn causes ubiquitination and degradation of Bim. Src-mediated activation of FAK enhances Bad phosphorylation by Akt, inhibiting caspases-2, -3, -8 and -9 and suppressing *anoikis* [8,23]. Moreover, compelling studies demonstrated that activation of Src due to its cysteine oxidation plays an important role in the induction of *anoikis* resistance in aggressive prostate cancers undergoing constitutive oxidative stress, mainly acting on EGF-R pro-survival signaling [89] (see Section 4.5).

Overexpression or constitutively activation of ILK further contributes to cancer malignancy, leading to anchorage and growth-factor independence, *anoikis* resistance, invasion of surrounding tissues and metastasis. In vivo, ILK is indirectly activated by PI3K, being its kinase activity strongly increased following interaction with phosphatidylinositol 3,4,5-triphosphate (PIP3). Conversely, PTEN, by hydrolyzing PIP3 to PIP2, contributes to downregulate ILK activity. Several evidence demonstrate that in epithelial cells, ILK overexpression or hyperactivation caused by PTEN loss of function or downregulation, are fundamental to achieve the mesenchymal phenotype [30,62,262,265]. Once activated, ILK acts on several pathways, including the small GTPases Rac1 and Cdc42, influencing cell spreading and migration, myosin light chain, thereby stimulating cell contractility and cell motility, as well as Akt phosphorylation, sustaining cell survival. On the other hand, ILK phosphorylates and inhibits GSK3, resulting in the stimulation of the activator protein 1 (AP1) and β-catenin/TCF transcription factors, which in turn increase synthesis of MMP-9 and cyclin D1, favoring tissue invasion and proliferation. Finally, ILK is able to increase Snail expression, thereby promoting EMT and *anoikis* resistance (see Section 4.3) [105].

An alternative mechanism to avoid *anoikis* consists in deregulated expression of growth factor receptors. As above described, their activation, also achieved through autocrine signaling of growth factors, triggers activation of cell survival pathways and stimulates cells migration and invasion. The neurotrophic tyrosine kinase receptor B (TrkB), frequently overexpressed in tumors, has been described as one of the most efficient in the induction of *anoikis* resistance [81,254]. TrkB is frequently overexpressed in many aggressive tumors and is also correlated to development of chemoresistance in gastric and prostate carcinomas [220,257]. Overexpression of TrkB causes changes in cells shape, causing rounding, detachment and induction of *anoikis* resistance, transforming nonmalignant cells into highly aggressive tumor cells [60]. In addition, TrkB triggers a clear EMT through the Twist–Snail–ZEB1 axis leading to sustained downregulating of E-cadherin expression [208]. TrkB is also active on PI3K and MAPK signaling pathways, concurring to both EMT and *anoikis* insensitivity.

It is widely described that signals triggered by integrins synergize with growth factors signaling pathways to modulate cell survival, as well as proliferation and migration. In mammary epithelial cells, loss of integrin-mediated adhesion leads to downregulation of EGF-R expression and inhibition of Akt and MAPK signaling, thereby leading to Bim accumulation and to the execution of the apoptotic program. Bypassing this anchorage requirement through growth factor receptor overexpression or their sustained activation is a typical mechanism adopted from cancer cells to escape from integrin control, thereby overcoming *anoikis*. Indeed, overexpression of EGF and ErbB2 receptors, a common feature of tumors, activates MAPK signaling inducing Bim degradation and blocking *anoikis* [201]. In human breast cancer, high level of Erb2B activity stimulates upregulation of α5 integrin, via the MAPK pathway, enhancing Src activation, which leads to sustain the ligand-independent Erb2B activation, as well as degradation of Bim, finally conferring *anoikis* insensitivity [95,100,181]. In some cases EGF-R is coupled with TGF-β1 signaling in the regulation of migratory and adhesive behavior, as both factors concur to activate FAK and Akt, contributing to overcome *anoikis* [109,229].

Finally, also overexpression of Neuripilin-1 has been correlated to *anoikis* resistance. Indeed, in pancreatic cancer cells Neuropilin-1 increases MAPK signaling and expression of the anti-apoptotic regulator Mcl-1, thereby enhancing survival of cancer cells in suspension [239].

4.3. Epithelial mesenchymal transition (EMT)

Epithelial mesenchymal transition (EMT) is a physiological process that allows epithelial cells to remodel cytoskeleton, release the

linkage with vicinal cells and acquire a motile phenotype. This phenomenon is usually activated during wound healing, inflammation or embryogenesis (Fig. 3A). EMT has also been described for cancer cells, allowing them to detach from neighboring cells, overcoming *anoikis* and to move from their primary location and invade others tissues. During EMT cancer cells activate epigenetic pathways that lead to the downregulation of cell–cell adhesion molecules, such as E-cadherins and γ-catenin, and, at the same time, to the expression of mesenchymal markers such as vimentin, fibronectin, α-smooth muscle actin (SMA), N-cadherin as well as to the activation of MMPs. It is known that the ability to overcome *anoikis* is correlated with the acquisition of the mesenchymal phenotype. This is possible because most of key players involved in EMT activation are able to modulate pro- and anti-apoptotic genes. Indeed, on one hand, they

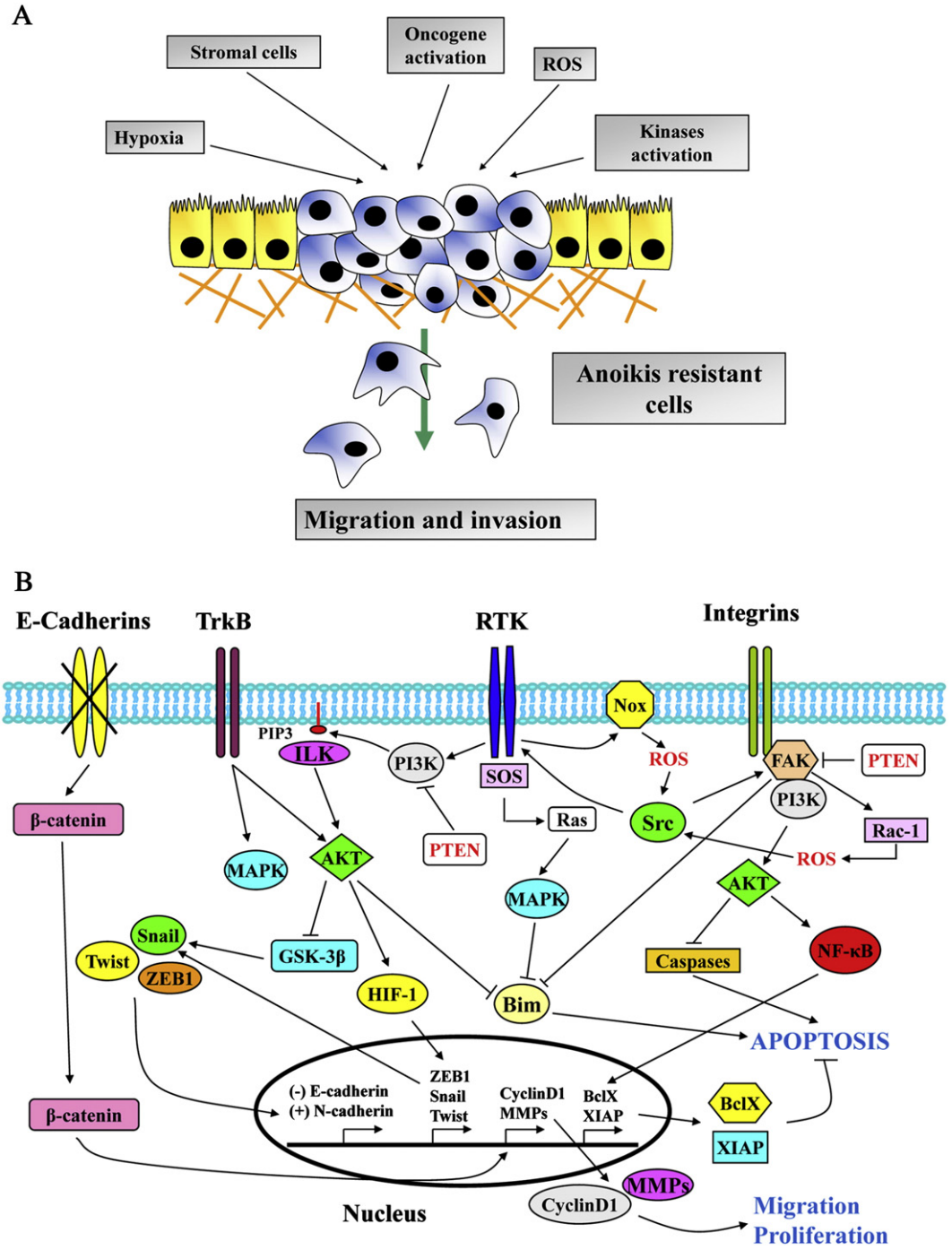

Fig. 3. EMT and *anoikis* resistance. (A) Stimuli that contribute to trigger EMT, allowing cancer cells to avoid *anoikis*. (B) Signaling pathways involved in the induction of EMT as well as in the *anoikis* resistance. Overexpression of RTKs, the change in the integrin pattern expression, downregulation of PTEN, all contribute to stimulate activation of pro-survival PI3K/Akt signaling pathway, inhibiting apoptotic program. On one hand, Akt acts directly, favoring degradation of proapoptotic proteins, while on the other hand, Akt leads to upregulation of both HIF-1 and NF-κB activities and the inhibition of GSK-3β, allowing the upregulation of Snail, ZEB1/2, Twist, and some of the master regulator of EMT. These, in turn, repress expression of pro-apoptotic proteins (Bid, Bax, Bim) and stimulate anti-apoptotic proteins expression (BclX/XIAP), contributing to overcome apoptosis. Increase of ROS production may also contribute to overcome *anoikis*, favoring the ligand-independent activation of growth factors or the redox-mediated downregulation of pro-apoptotic factors. The downregulation of E-cadherin expression elicits β-catenin migration into the nucleus, where it stimulates the expression of target genes involved in the regulation of cells motility and invasion, such as c-Myc, cyclin D1, c-Jun, MMP-1 and MMP-7.

upregulate the expression of anti-apoptotic genes (Bcl-2 family) and/or activate pro-survival pathways (i.e. PI3K/Akt), while on the other hand, they lead to downregulation of pro-apoptotic proteins such as p53-effector related to pmp22 (PERP), p21, Bim, Bax and Noxa [246].

Key players involved in EMT induction are transcription factors such as Snail, ZEB1/2, Twist, NF-kB and HIF1/2 (Fig. 3B). They are often aberrantly expressed in cancer cells, and share the ability to decrease E-cadherin expression, while increasing the expression of mesenchymal markers. For example, Twist activation strongly contributes to migration and invasion, as confirmed by the evidence that its downregulation reduces both processes. On the other hand, Twist promotes survival, upregulating the level of anti-apoptotic Bcl-2 protein [136,250]. Similar mechanism has been described for Snail-1, which has found upregulated in primary human breast carcinomas and breast tumors [19]. In particular, several spurs, including insulin-like growth factor and hepatocyte growth factor/scatter factor, inhibit glycogen synthase kinase-3β (GSK-3β), thus inducing the ubiquitin-mediated Snail degradation. As a consequence, Snail directly represses E-cadherin transcription as well as of other genes involved in *anoikis*, such as Bid, caspase-6, or PTEN. Downregulation of PTEN leads to PI3K/Akt pathway activation, favoring phosphorylation and inactivation of the pro-apoptotic protein Bad, thus contributing to *anoikis* resistance [11].

ZEB1 transcription factor has been associated to anchorage-independent growth of lung cancer cells, contributing to EMT and malignancy [82,164,219]. Again, ZEB1 expression causes an increase of Vimentin and a decrease of E-cadherin and semaphorin 3F expression, events that contribute to activation of Akt pathway, thereby promoting *anoikis* resistance [219].

The downregulation of E-cadherin expression promotes cytoplasmic accumulation of free β-catenin, which migrates into the nucleus leading to upregulation of target genes involved in the regulation of cells motility and invasion, such as c-Myc, cyclin D1, c-Jun, MMP-1 and -7 [196] (Fig. 3B). Overexpression or cytoplasmic stabilization of β-catenin, due to mutations affecting its degradation, confers *anoikis* resistance to cancer cells through the involvement of MAPK, c-Myc, and cyclin D1, and maintains a stable mesenchymal phenotype, repressing genes of the epithelial signature [165].

Other important transcription factors contributing to *anoikis* resistance of cancer cells are hypoxia-inducible factors (HIFs). Cancer cells proliferate quickly, thereby generating poorly vascularized tumor masses characterized by hypoxic or anoxic regions. The activation of HIF-1/2 is instrumental for cancer cells to trigger the EMT program, allowing them to escape from the hostile hypoxic milieu [126,228]. In particular, the role of HIF1 in sustaining *anoikis* resistance in both mammary or prostate cancers, is mainly linked to its ability to drive EMT, by promoting Twist or NF-kB activation and sustaining Snail expression [86,87,215]. Some evidence indicate that HIF-1 may also lead to *anoikis* protection through enhanced EGFR expression, activation of MAPK and causing degradation of pro-apoptotic proteins such as Bim and Bmf [241]. In keeping with the key role played by HIF-1 in the regulation of metabolism of cancer cells, it has been described that prolonged hypoxia increases the expression of several proteins involved in the control of autophagy, such as BNIP3, Beclin-1 and ATG5, suggesting that HIF-1α can also metabolically sustain *anoikis* resistance by modulating autophagy [256]. Beside the hypoxic activation of HIF-1, the transcription factor can also be activated independently from decrease of oxygen, for example in response of activation of membrane receptors. Normoxic stabilization of HIF-1 in response to ErbB2 expression by cancer cells is due to constitutive activation of Akt, able to stimulate HIF-1 α and β subunits association, thereby upregulating HIF activity in a hypoxia-independent manner. In keeping, depletion of HIF-1α in cancer cells restores *anoikis* sensitivity in detached cells, whereas does not affect cell death of ECM attached cells [143]. Of course, sustained stimulation of several other growth factor receptors such as EGFR, IGF-1R, stem cell factor receptor, TGF-βR and Notch, may behave similarly with respect to ErbB2, eliciting PI3K/Akt activation and enhancing HIF-1 activity, modulating the expression of EMT and anti-apoptotic genes. Indeed, beside Snail, Twist or NF-kB, HIF-1 activation modulate expression of other EMT effectors, such as CXC chemokine receptor 4 (CXCR4) and its ligand stromal derived factor-1, as well as of stemness/pluripotency-associated transcription factors such as Oct-3/4, Nanog and Sox-2 [156].

NF-κB is an important redox-sensitive transcription factor implicated in the regulation of development, inflammation, cell proliferation and survival. In its inactive form NF-κB binds IκB and is retained in the cytosol. Several stimuli can induce the phosphorylation and dissociation of IκB from NF-κB, which migrates into the nucleus and induces expression of anti-apoptotic proteins, such as Bcl-xL and XIAP [226]. NF-κB is constitutively activated in several cancer type and strongly contributes to activate and maintain cancer cells in a mesenchymal state through engagement of the EMT platform [86,87,245]. Once activated, NF-κB is able to regulate *anoikis* resistance through activation of the pro-survival PI3K/Akt signaling pathway, repressing pro-apoptotic proteins, as well as activating Snail-1, MMP-2 and -9, interleukin-8, vascular endothelial growth factor and CXCR4, coupling *anoikis* resistance to metastasis dissemination [106,112,124].

4.4. microRNAs

MicroRNA (miR) are non coding RNA that post-translationally regulate gene expression [78]. Compelling evidence demonstrate that several miRs are directly involved in negative EMT regulation and acquisition of *anoikis* resistance, even if they can exploit different pathways. The majority of miRs is downregulated as tumors become less differentiated and malignancy increases, suggesting a role for these molecules in determining cellular differentiation state and cancer aggressiveness [91].

miR200 family is surely the most acknowledged to be involved in the regulation of the epithelial phenotype. The family includes miR-200a, -200b, -200c, -141 and -429. Most of these repress ZEB1/2, upregulating E-cadherin expression and driving mesenchymal epithelial transition (MET), the reversal epigenetic plasticity adaptation with respect to EMT [212]. On the other hand, ZEB1/2 repress miR-200 family expression, thus contributing to generate a negative feedback loop with the miR-200 family [107]. Analyses carried out on several cancer cells highlight that downregulation of expression of miR200 family members is strictly related to EMT (Fig. 4). For example, expression of miR200c in breast cancer cells leads to EMT inhibition [227]. Beside its regulation of ZEBs, miR200c acts on the modulation of TrkB, thereby inhibiting it: cell transfection with a mutant of TrkB, insensible to miR200c regulation, stimulate EMT, promoting *anoikis* resistance [111]. Finally, forced expression of miR-200c in human endometrial carcinoma cells reduces migration, invasion and increases sensitivity to taxanes [207]. In agreement with this, miR-200c has often been found downregulated in several histotypes of cancer, allowing upregulation of several genes such as these encoding fibronectin-1, moesin, TrkB, leptin receptor and Rho GTPase activating protein-19 [111]. The consequence of miR-200c downregulation is the activation of signaling pathways that stimulate cell motility, EMT and *anoikis* resistance [260]. Moreover, beside their role in EMT modulation, miR200 family members are also able to induce *anoikis* resistance in cancer cells using different routes. Indeed, it has been observed that in human breast cancer cells, overexpression of miR-200a promotes *anoikis* resistance through the regulation of the anti-apoptotic protein YAP1, with a clear correlation with metastasis diffusion in patients with breast cancer [252].

Besides miR200s, other miRs have been negatively correlated to *anoikis* resistance, and again their activity appear linked to EMT control (Fig. 4). For example it has been demonstrated that miR-155, downregulating RhoA, is important to induce TGF-β mediated EMT and *anoikis* resistance [131]. In addition, miR-30a has been identified

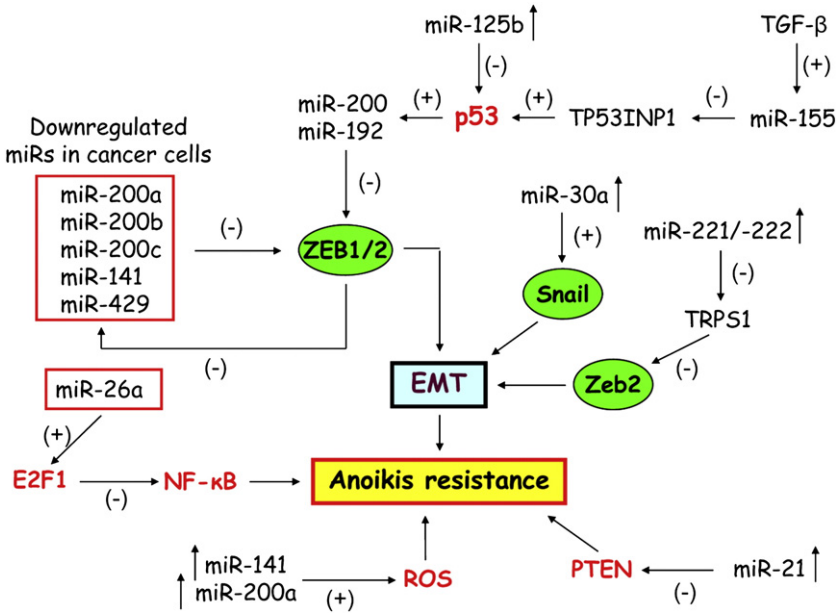

Fig. 4. Role of miRNA in the induction of *anoikis* resistance. Several miRNA are involved in the control of cell differentiation and their expression inhibits EMT and promotes *anoikis* in detached cells. In cancer cells, their downregulation favors cell survival. For example, downregulation of miR-200a, -200b, -200c, -141 and miR-429 stimulates expression of transcription factors ZEB1/2, promoting EMT and *anoikis* resistance. On the other hand, decrease of miR-26 expression causes a RB1-mediated E2F1 inhibition, thereby leading to NF-κB activation, EMT induction and contributing to *anoikis* resistance. On the contrary, in several cases, it has been observed that the overexpression of miRNA, more than their downregulation, is involved in the control of EMT process. For example, the increase expression of miR-125b, as well as TGF-β-dependent expression of miR-155, leads to downregulation of p53, which controls the expression of both miR-200 and miR-192 families, known repressors of ZEB1/2. Thus, expression of both miR-125b and miR-155 activates EMT, contributing to enhance detached cells survival. Similarly, upregulation of miR-221/222 contributes to ZEB2 expression and EMT induction; furthermore, upregulation of miR-30a regulates EMT increasing expression of Snail. Other miRNA lead to *anoikis* resistance through different mechanisms. Upregulation of both mir-141 and miR-200a increases ROS production, activating pro-survival pathways. Finally, expression of miR-21 downregulates PTEN expression, activating pro-survival PI3K/Akt signaling pathway.

as a regulator of Snail, and its role in *anoikis* resistance is very likely, although not yet addressed [133]. Similarly, as recent studies suggest that p53 suppression of EMT is mediated by miR200 and miR192 family members through repression of ZEB1/2, we can speculate that miR192 family can also be involved in the regulation of *anoikis* insensitivity [96,129].

Some miRNA are implicated also in the regulation of ROS production and modulation of oxidative stress, a mandatory prerequisite for EMT [86,87,177]. For example, it has been observed that high-grade human ovarian adenocarcinoma cells express both miR-141 and miR-200a and show low p38α activity and are characterized by an associated oxidative stress signature [152]. Compelling evidence demonstrate that in cancer cells oxidative stress promotes EMT (see Section 4.5), thereby suggesting that both miR-141 and miR-200a may promote *anoikis* resistance through EMT-oxidative stress mediated induction.

Overexpression of miR-221 and miR-222 protects cancer cells from apoptotic stimuli induced by several drugs or from *anoikis* after cells detachment. Both miR-221 and 222 contribute to induce EMT by targeting the 3′ untranslated region of trichorhinophalangeal syndrome type 1, a transcriptional repressors that inhibits EMT by reducing ZEB2 expression. Thus, miR-221 and miR-222, increasing ZEB2 activity, may stimulate EMT, overcoming *anoikis* [214].

Beside the regulation of EMT, miRs can regulate *anoikis* resistance by directly controlling pro-survival pathways. In this view, mir-21 has been reported to target PTEN and the pro-apoptotic programmed cell death 4 antigen (PDCD4), leading to downregulation of PTEN, stimulation of Akt pathway and reducing apoptosis in cancer cells and promoting cell survival [116].

miR-210 has been correlated to the transcriptional program engaged by hypoxia. This miR has been found expressed in many tumors and its contribution to inhibition of pro-apoptotic signaling in an hypoxic environment is linked to its dependence from HIF-1 [37]. miR-155 targets the tumor protein p53 inducible nuclear protein 1 (TP53INP1), a positive regulator of p53-mediated apoptosis. As expected, downregulation of TP53INP1 by miR-155 attenuates apoptotic pathway, promoting cell survival [93].

miR-26a is downregulated in human esophageal adenocarcinoma cells, leading to increased Rb1 expression levels and causing E2F1 inhibition, thereby inducing cell cycle arrest. As E2F1 is involved in the repression of NFκB activity, the consequence of miR-26a downregulation are both NFκB activation and *anoikis* suppression [260]. Moreover, in mesenchymal stem cells, expression of miR-125b increases MAPK phosphorylation and suppresses p53 expression, promoting cell survival in response to detachment [253].

Nevertheless our understanding about the role of miRs in cellular functions is at its infancy, their downregulation is a common feature of cancer cells and this correlates with cancer cell plasticity and stemness through the regulation of MET/EMT, thereby affecting cancer cell resistance to *anoikis*.

4.5. Regulation of anoikis resistance due to oxidative stress or hypoxia

Compelling evidence demonstrate that in cancer cells chronic ROS production contributes to promote cell survival, proliferation and metastatic dissemination. External factors, such as exposition to radiation, chemicals or drugs can contribute to increase of intracellular ROS levels. Moreover, activation of cellular receptors, EMT engagement and p53 inactivation may contribute to ROS production [90] (Fig. 5).

Exposure of lung carcinoma and melanoma cells to subtoxic doses of hydrogen peroxide significantly upregulates Cav-1 which, by activating Akt pathway, leads to *anoikis* resistance and anchorage-independent growth [102]. Similarly, treatment with hydrogen peroxide abrogates *anoikis* commitment after cell detachment, by preventing Cav-1 downregulation [185]. Exposure of human lung carcinoma cells to subtoxic doses of cisplatin, chronically increases intracellular ROS levels and Cav-1 expression, leading to *anoikis* resistance [210].

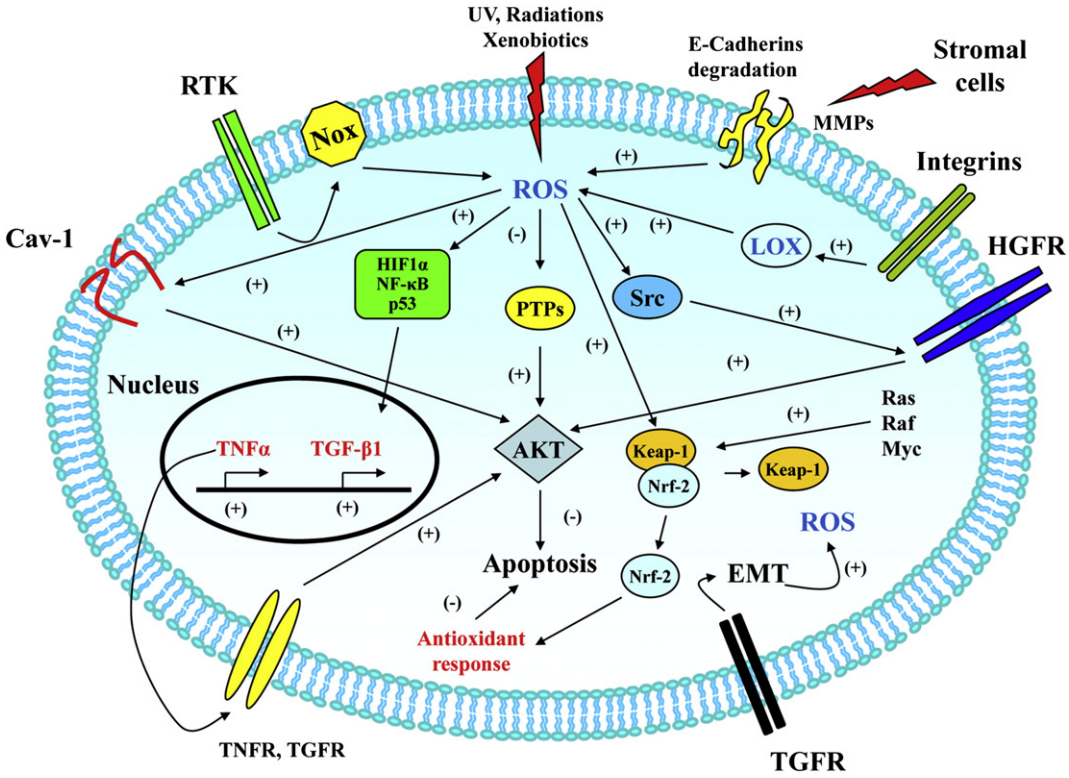

Fig. 5. Alteration of cancer cell redox state and acquisition of *anoikis* resistance. Several stimuli, such as RTK overactivation, UV, radiations, drugs and xenobiotics, integrin engagement, cytokines and growth factors, contribute to the increase in intracellular ROS levels. These, in turn, activate redox-sensitive transcription factors (HIF-1, NF-κB and p53) promoting the increase of expression of anti-apoptotic proteins (Bcl-xL, XIAP, TRAF1 and c-FLIP), or the suppression of pro-apoptotic protein such as Bim and Bmf. Activated transcription factors stimulate also the expression of TNFα and TGF-β1, thereby sustaining cancer cells autocrine stimulation loops. ROS inhibit PTPs, increasing pro-survival PI3K/Akt signaling pathway, thereby leading to inhibition of pro-apoptotic pathways. In addition ROS activate Src kinase which sustains ligand-independent EGFR activation, leading to *anoikis* resistance. ROS mediated Nrf-2 activation allows cancer cells to control intracellular ROS levels. Once activated, Nrf-2 migrates into the nucleus and stimulates expression of several antioxidant enzymes. Overall, these mechanisms enable cancer cells to adapt to stress condition, overcoming *anoikis*.

Activated growth factor receptors increase intracellular ROS production by activating enzymes such as NADPH oxidase (Nox) and lipoxygenase. Most cancer cells overexpress growth factor receptors or show autocrine behavior, producing and secreting growth factors that sustain receptor activity and constitutive ROS production. Notably, ROS modulates activation of Akt and MAPK signaling pathways, as well as the activity of redox-sensitive transcription factors (NF-kB, HIF-1/2, p53, AP-1, Nrf2, etc.), thus contributing to sustain autocrine loops [97]. It has also been observed that growth factor activation increases Nox expression, contributing to maintain high levels of ROS production [33].

Increase of ROS levels is important to achieve *anoikis* resistance in cancer cells. The activity of most proteins involved in signaling pathways activated by both integrins and growth factor receptors are regulated by reversible phosphorylation on serine, threonine or tyrosine residues. Phosphotyrosine phosphatases are susceptible to oxidative modification of essential catalytic cysteine residue, thereby causing their oxidative inhibition [46]. Several studies showed that sustained ROS production leads to constitutive inactivation of PTEN, PTP-1B, SHP2, LMWPTP, PP2a and PP1a enzymes. In addition, ROS promote the activation of Src kinase and several redox sensitive transcription factors (NF-kB, HIF-1α, p53 AP-1) [168], contributing to sustain PI3K/Akt signaling pathway and enhance cell survival through pro-apoptotic Bad inhibition [94,171].

It has been demonstrated that in human epithelial cells ROS activate Src kinase, which transactivates EGFR in a ligand-independent manner. This activates both MAPK and Akt signaling pathways, leading to degradation of the pro-apoptotic protein Bim [88]. Moreover, angiopoietin-like 4 protein interacts with β1 and β5 integrins and stimulates superoxide production through Nox activation, thus mimicking anchorage conditions and bypassing *anoikis* by controlling ROS [222]. In addition, moderate increase of ROS leads to NF-κB activation promoting the increase of expression of anti-apoptotic proteins such as Bcl-xL, XIAP, TRAF1 and c-FLIP, the inhibition of JNK, and the upregulation of antioxidant genes such as Mn-SOD. By these alternative pathways, cancer cells rescue the balance of ROS levels and become insensitive to apoptosis [125].

Constitutive oxidative stress may also affect *anoikis* insensitivity in strict correlation with malignancy. Aggressive and metastatic prostate cancer cells undergo a constitutive activation of 5-lipoxygenase, sustaining increased intracellular ROS. These, in turn, oxidize and activate Src kinase enhancing the ligand-independent EGFR activation. In turn, sustained EGFR signaling leads to inhibition of Bad phosphorylation and Bim degradation, thereby promoting cell survival even in the absence of adhesion to ECM. Antioxidant treatment of prostatic cancer cells completely abolishes the ligand-independent activation of EGFR, as well as their resistance to *anoikis*, thus restoring the apoptogenic stimuli [89].

Rapid growing tumors exhibit hypoxic intratumoral regions and require a complex adaptation of cancer cells for their survival. Briefly, hypoxia activates a transcriptional response leading cancer cells to activate i) a glycolytic metabolism sustaining survival, ii) an escaping strategy through enhanced motility and iii) secretion of angiogenic growth factors to reconstitute a functional vasculature. Hypoxia promotes EMT in a variety of carcinoma cells, including melanoma, breast, prostate and colon cancers [115,139]. In detached hypoxic cells, *anoikis* inhibition occurs through HIF-1 dependent upregulation of Snail and Twist and suppression of pro-apoptotic protein such as Bim and Bmf (also see Section 4.3) [115,241]. On the other hand, it has been observed that hypoxia leads to increase of intracellular ROS leading to inhibition of both prolyl hydroxylase and asparagyl hydroxylase, the most important negative regulators of HIF-1 [240].

These events contribute to HIF-1 stabilization, allowing it to regulate the expression of genes involved in cell survival, metabolism as well as motility and invasion. At the same time ROS production contributes to inhibit GSK-3β, nuclear Snail translocation, E-cadherin downregulation, thereby activating the EMT program and sustaining *anoikis* resistance [101] (Fig. 5).

Cancer cells respond to hypoxia activating several mechanisms that allow them to survive and even proliferate in a hypoxic environment. It is likely that the pro-survival pathways activated during hypoxia and already linked to chemotherapy resistance in several cancer models, can also contribute to *anoikis* resistance in detached hypoxic cancer cells. The role of hypoxia in granting a pro-survival spur is clear. For example, hypoxia protects hepatoma cells against etoposide-induced apoptosis, downregulating p53 expression and increasing c-Jun DNA binding activity [50,199]. Similar results has been obtained with breast cancer cells treated with paclitaxel. In this case, hypoxia was able to increases the expression of c-Jun and DNA binding activity of AP-1. In turn, c-jun was able to upregulate Mcl-1 expression which participates to the hypoxia-induced protection against apoptosis induced by paclitaxel [66]. Severe hypoxia or anoxia leads to HIF-1-independent expression of the anti-apoptotic protein IAP-2 that protects cells from apoptosis [59]. Mild hypoxia protects cells prom apoptosis interfering directly with several components of the apoptotic pathway and downregulating the expression of nearly all the pro-apoptotic Bcl-2 family proteins, decreasing Noxa and Bad abundance or leading to post-translational modification of Bim, enhancing cell survival and inducing chemoresistance [200,241].

Several tumors also show an increased antioxidant capacity in response to oxidative stress, suggesting that enhanced antioxidant activity is necessary for tumor progression. To avoid *anoikis* triggered by excessive ROS production, detached cancer cells are able to regulate expression of several antioxidant enzymes through a mechanism that involve the transcription factor Nrf-2. Normally, Nrf-2 binds Keap-1 and is retained therefore in the cytoplasm. When ROS concentration increases over the threshold, leading to Keap-1 oxidation, Nrf-2 dissociates from oxidized Keap-1 and migrates into the nucleus enhancing expression of many antioxidant proteins, including heme oxygenase-1, peroxiredoxin-1, the heavy and light chains of ferritin, catalase, glutathione peroxidase, superoxide dismutase, and thioredoxin [142]. This transcriptional control allows survival of cells in prooxidant milieu, a very common feature of tumor cells and their microenvironment [167]. Nrf-2 has an important role in preventing cancer cells death, as it has been identified as an inhibitor of Fas-induced apoptosis. Growing cancer cells in the presence of glutathione, leads to inhibition of cells death, suggesting that the anti-apoptotic effect of Nrf-2 was through elevating intracellular glutathione levels [157]. It is interesting to note that several oncogenes, such as Ras, Raf and Myc, may activate the Nrf-2 pathway to protect cancer cells from the oncogene-addicted oxidative stress [226]. In keeping, Nrf2 upregulation is important to reduce in ECM-detached cancer cells intracellular ROS concentration enhancing cell survival and leading to *anoikis* resistance [34]. This hypothesis is confirmed by the evidence that genetic targeting of the Nrf-2 pathway in K-Ras overexpressing murine cells impairs proliferation and cell survival [57].

Moreover, Nrf-2 activation is not the unique mechanism allowing cancer cells to compensate ROS production. For example, H-Ras-transformed cells, express elevated levels of peroxiredoxin-1 and thioredoxin peroxidase with respect to parental non tumoral cells [34]. Moreover, in melanoma cells, c-Myc exerts its pro-survival role through upregulation of GSH production [18]. Interestingly, inhibition of manganese superoxide dismutase reverts chemoresistance to taxanes [178], suggesting that similar mechanisms may also play a role in conferring *anoikis* resistance to cancer cells.

In addition ROS production is correlated to EMT [90]. For example, it has been reported that TGF-β was shown to induce EMT via upregulation of hydrogen peroxide and MAPK signaling, whereas both ROS production and Snail activation are requested in mammary epithelial cells for MMP-3-mediated EMT [177]. Recently, it has been demonstrated that prostate cancer cells expressing Snail undergo EMT and displayed increased concentration of ROS. Cells treatment with both reducing agent or MEK inhibitor partially reverts Snail-mediated EMT, demonstrating that Snail regulates oxidative stress enzymes and increase ROS-mediated EMT regulated in part by MAPK activation [10]. Finally, recent studies highlight the role of tumoral microenvironment in the induction of oxidative stress and EMT in cancer cells. Cancer associated fibroblast release MMP-2 and -9 leading to E-cadherin cleavage and Rac1b/cyclooxygenase-2-mediated release of ROS which, in turn, trigger EMT, thereby contributing to induction of *anoikis* resistance [86,87].

4.6. Avoiding anoikis by modulating energetic metabolism

Recently, it has been observed that cells detachment from ECM strongly influences metabolism of normal cells, reducing glucose uptake, glycolytic flux, mitochondrial respiration and the pentose phosphate pathway. The consequences of detachment from ECM are the reduction of both intracellular ATP and NADPH concentration, reduction of fatty acid oxidation, increase of ROS production and induction of apoptosis. ErbB2 overexpression is enough to restore glucose uptake through PI3K/Akt pathway activation, quench ROS increasing NADPH production, rescuing cells from *anoikis* [35,189]. In the last years it has been highlighted that in cancer cells modulation of metabolic pathways contribute to achieve an *anoikis* resistant phenotype. Cancer cells metabolize high glucose levels through glycolysis, but most of pyruvate obtained is transformed into lactate instead of being oxidized in mitochondria, a phenomenon described as the Warburg effect. Glucose uptake reduction in response to cell matrix detachment activates large kinase B1 (LKB1) which, in turn, increases AMPK activity which modulates *anoikis*. Indeed, once activated, AMPK inhibits acetyl-CoA carboxylases 1 and 2, lowering NADPH consumption in fatty-acid synthesis, but increasing NADPH generation through an alternative pathway fuelling fatty-acid oxidation. This mechanism is essential to reduce ROS produced after matrix detachment, avoiding *anoikis* and eliciting cancer cells to survive during the early stages or tumorigenesis or during migration [120] (Fig. 6).

Others key players of cancer metabolic reprogramming are HIF-1, c-Myc, PTEN, and p53. Usually HIF-1 acts promoting expression of genes involved in the regulation of several biological processes, including cell proliferation, angiogenesis, metabolism, immortalization, migration but also apoptosis. The fate of cells depends on the balance of these pathways, leading to death or cell survival. Moreover, it has been observed that HIF-1α is phosphorylated and stabilized also through oncogenic signaling pathways involving Src, Ras, protein kinase C, and PI3K, explaining why in cancer cells it has been often found overexpressed and activated. Hence, HIF-1 is mandatory to deeply reprogram cancer metabolism in terms of increase of nutrient uptake, in both hypoxic and normoxic conditions. Indeed, HIF-1 stimulates expression of glucose transporter GLUT1-3 leading to increased glucose uptake [40]. In addition, HIF-1 directly forces Warburg metabolism by transcriptional regulation of glycolytic enzymes such as hexokinase-2, pyruvate dehydrogenase (PDH) kinase-1 (PDK-1), lactate dehydrogenase-A and pyruvate kinase isoform-2 (PK-M2), a low-active splice form with respect to pyruvate kinase isoform-1, which is inhibited by oxidative stress or by phosphorylation on tyrosine residue [28,58,251]. Interestingly, it has been observed that PK-M2 hydroxylation leads to nuclear translocation and stimulates binding and activation of HIF1α, increasing the expression of metabolic enzymes under hypoxia, thereby originating a positive loop enhancing Warburg effect [149]. Likewise, c-Myc upregulates GLUT1, lactate dehydrogenase A, hexokinase 2, phosphofructokinase and glutaminase-1, the first enzyme of glutaminolysis [52]. Another important key player in cancer metabolic reprogramming is p53, which regulates glycolysis, PPP, oxidative phosphorylation and glutaminolysis. In normal cells, p53 directly inhibits glucose-6-phosphate dehydrogenase, downregulating NADPH

production, inhibits expression of GLUT1, 3 and 4, inhibits glycolysis, upregulating expression of TIGAR and Parkin proteins, whereas stimulates oxidative phosphorylation and glutaminolysis. In addition it has been observed that p53 increases expression of PTEN and AMPK [65,213]. Loss of function of p53 or its downregulation leads to reversion of all above mentioned metabolic effects, stimulating uptake of glucose and glycolysis, but decreasing mitochondrial oxidative phosphorylation and contributing to sustain Warburg effects [153].

Compelling evidences indicate that this metabolic adaptation meets with the necessity of cancer cells to maintain non apoptogenic ROS levels. In the presence of ROS PK-M2 is converted in a fully inactive form, behaving as a bottleneck for glycolysis and promoting NADPH synthesis through PPP, contributing to decrease ROS levels and leading to *anoikis* resistance [3]. Accordingly, it has been demonstrated that low pyruvate kinase activity leads to increased PEP which, in turn, inhibits trioso-phosphate isomerase, blocking glycolytic flux and redirecting glucose-6-P in the PPP. By this way, cells increase NADPH synthesis and prevent an increase in ROS upon activation of respiration, sustaining cancer cell survival and likely promoting *anoikis* resistance [99].

Finally, it has been observed that upon detachment, human mammary cells upregulate pyruvate dehydrogenase kinase 4 (PDK4) that inhibits PDH, reducing mitochondrial respiration, lowering ATP production and stimulating glycolytic flux. PDK4 has an important role in the induction of *anoikis* resistance as the stimulation of PDH activity leads to increased *anoikis* sensitivity and to impair metastatic potential of cancer cells [122].

Of course the ability of cancer cells to exploit autophagy in response to detachment (see Section 3.4) is strongly correlated to their metabolic deregulation. Autophagy forces tumor cells into dormancy, allowing them to survive hostile conditions, remaining ready to de novo reprogram metabolism upon environmental/nutrition improvement avoiding death [26,211]. In keeping with this view, autophagy is regulated by nutrient deprivation and therefore by hypoxia/ischemia, oxidative stress, TRAIL and AMPK in several cancer models [6,44,104,172]. In addition, several oncogenes sustains EGFR expression in cancer cells, enhancing antioxidant capacity, enhancing glucose uptake and fatty acid oxidation, fuelling cells with ATP and granting survival to *anoikis* [147,189]. In keeping, in breast ductal carcinoma the endoplasmic reticulum kinase PERK facilitates survival of ECM-detached cells by concomitantly promoting autophagy, ATP production, and an antioxidant response [7]. As a final point, autophagy has an essential metabolic role ensuring cancer cell survival by eliminating dysfunctional mitochondria, allowing Warburg metabolism [259]. Of note, autophagy is triggered by accumulation of ROS, due to mitochondrial failure [148,258].

5. Cancer cells exploit *anoikis* resistance in their long metastatic route

Cancer progression towards malignancy consists of multiple steps which can induce or facilitate metastatic spread of tumor cells to distant organs and reconstitution of metastatic colonies. This "long metastatic route" can be mainly categorized into these steps: 1) carcinogenesis in the primary site, 2) sustained proliferative signaling and hyperproliferation of cancer mass, 3) generation of hypoxic environment inside the cancer bulk, 4) sustained angiogenesis/lymphangiogenesis to reconstitute the adequate supply of oxygen and nutrients, 5) cross-talk with the component of the new microenvironment including parenchymal, stromal, endothelial and inflammatory cells, 6) migration through the extracellular matrix and invasiveness, 7) intravasation into the bloodstream 8) cell survival in the blood and lymphatic vessels, 9) extravasation from the circulation into the surrounding tissues of distant organs, 10) preparation of the metastatic niche in which cancer cells should adapt, and 11) growth of the invading cells in the new site [137,151].

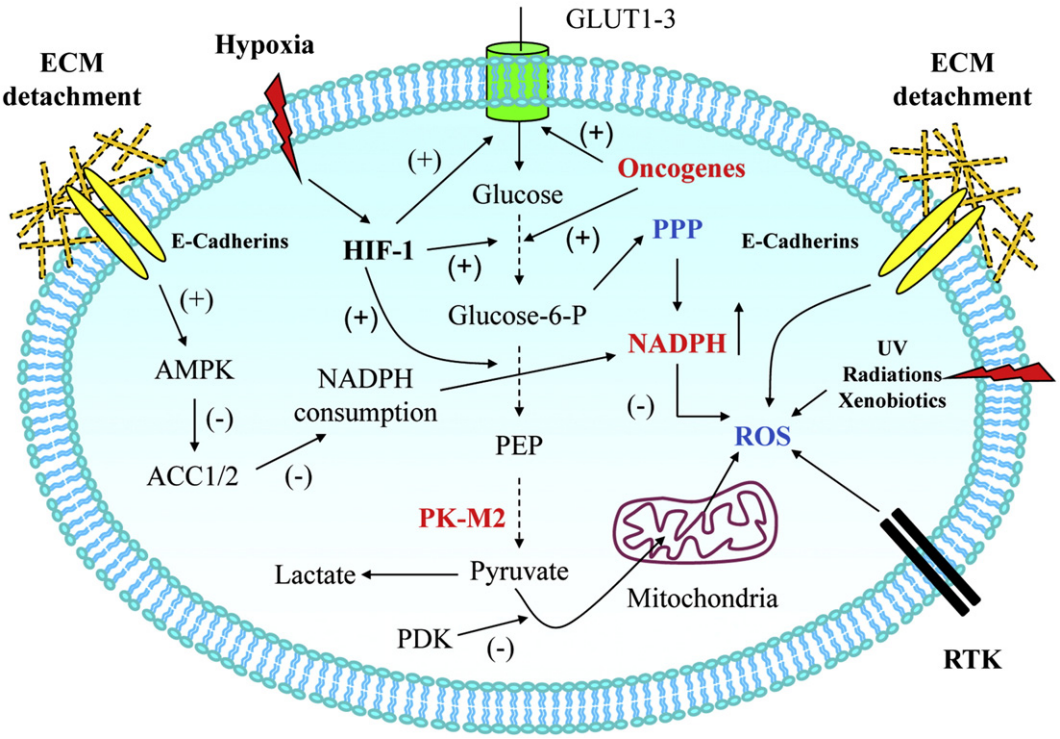

Fig. 6. Modulation of *anoikis* by metabolic pathways. Cell detachment, receptors and oncogene activation, hypoxia, radiation or xenobiotic agents upregulate ROS production, increasing the risk of cell death. Moreover, metabolic reprogramming contributes to inhibit *anoikis*. Oncogenes, as well as HIF-1, enhance expression of glucose transporters, glycolytic enzymes, PDK and PK-M2, strongly increasing the glycolytic flux, but inhibiting the oxidative phosphorylation. This forces cancer cells to increase NADPH production through PPP, in order to reduce ROS levels, avoiding *anoikis*. Glucose uptake reduction in response to cell matrix detachment increases AMPK activity, which inhibits acetyl-CoA carboxylases 1 and 2, lowering NADPH consumption in fatty-acid synthesis. This mechanism allows to handle oxidative stress in response to matrix detachment, thereby avoiding *anoikis*.

Anoikis resistance of cancer cells plays a pleiotropic role during several of these steps and for this reason it becomes an attractive pharmacological target for anti-metastatic therapies (Fig. 7).

First of all the ability of cancer cells to resist natural apoptotic death when non adherent or adherent to improper matrix is a guarantee to obtain the increase in cancer mass, mainly due to sustained autocrine secretion of cytokines and growth factors. Indeed, the increased size of the mass of cancer cells grossly eliminates the interaction among cancer cells and their surrounding matrix. Within the first phases of carcinogenesis the apoptosis inside the cancer mass is massive and it is likely that the improper or absent adhesion to ECM plays a mandatory role in their apoptosis via *anoikis*. The achievement of *anoikis* resistance due to genomic instability will surely sustain survival of cancer cells, thereby enhancing malignancy. Moreover, *anoikis* escaping cells also activate self-cannibalism through autophagy, mainly mediated by the PERK/AMPK signaling and specifically activated by de-adhesion and not by endoplasmic reticulum stressors, thereby granting for luminal filling during early carcinoma progression [6]. In parallel to autophagy, cancer cells during their progression towards malignancy are profoundly reprogrammed in their metabolism, undergoing changes leading to a Warburg phenotype. Of note, many of the metabolic changes concerning Warburg behavior are mediated by the same pathways allowing survival to *anoikis*, as Akt activation, p53 loss of function or HIF-1 stabilization [237]. For example in breast cancer cells the detachment from ECM reprograms their metabolism towards Warburg-like phenotype, leading to pyruvate kinase inhibition and divergence of glucose from respiration. In keeping with this idea, decrease of glucose respiration confers *anoikis* resistance to breast cancer cells [123].

Beside decrease of cell–matrix contact, tumor progression within the primary site is accompanied by decreased cell–cell contact and elimination of adherens junctions among cancer cells. This process is mainly due to the activation of EMT, leading to E-cadherin repression and/or cleavage and elimination of cadherin-dependent cell–cell contact, as well as de novo expression of N-cadherin, which favors per se *anoikis* resistance. EMT can be either due to genetic intrinsic features of cancer cells or to interaction with environmental cues, including hypoxia or interaction with stromal cells. Indeed, cancer associated fibroblasts have been reported to enhance EMT in cancer cells within the primary site and increase their metastatic potential through regulation of stem-cell traits. Besides, intratumoral hypoxia has been described as one of the environmental factors engaging EMT, mainly acting through stabilization of the hypoxia inducible transcription factors (either HIF-1 or HIF-2) and activation of a motogen program executed by the Met kinase, granting for invasive escaping from the hostile hypoxic tumor site. Of note, *anoikis* resistance has been clearly correlated to EMT engagement and the transcriptional programs leading to EMT, through Snail-1, Snail-2, Twist, Nf-κB ZEBs, HIF-1, HIF-2 transcription factors, also activates pathways leading to evade *anoikis* by constitutively activating specific pro-survival signals. For example, Snails and ZEBs inhibit the transcription of E-cadherin and confers apoptosis resistance by activating survival genes such as the PI3K/Akt pathway. In keeping, loss of E-cadherin in mammary tumorigenesis models grants for *anoikis* resistance and increased angiogenesis, thus contributing to efficient metastatic spread. Furthermore, key executors of the EMT program, like Met proto-oncogene or Trk kinase, have also been reported to enhance the resistance to *anoikis* of cancer cells, thereby confirming the strict correlation between resistance to loss of adhesion and motility through EMT [15]. In keeping with the ability to confer resistance to *anoikis*, EMT has also been related with resistance to both radiation and treatment with chemical agents, in strict correlation with the acquisition of stem and survival features [43,134]. This is related to the inactivation of p53-mediated apoptosis, promoted by Snail-1, Slug, or Hedgehog signaling [134].

Beside EMT another adaptation in motility style has been reported as mandatory for cancer cells in order to metastasize: mesenchymal

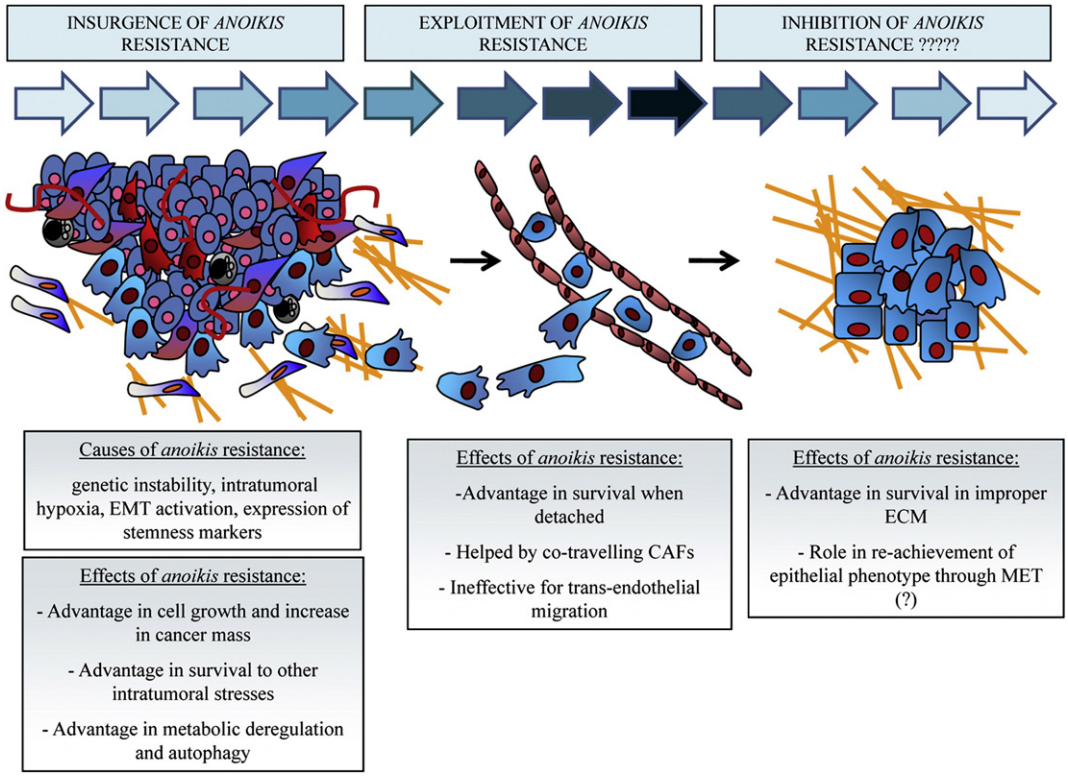

Fig. 7. Cancer cells exploit *anoikis* resistance in their long metastatic route. The cartoon illustrates the metastatic route run by malignant cancer cells, starting from the primary tumor, alongside circulation and culminating in metastatic colonization of distant organs. *Anoikis* resistance emerges mainly within the primary tumor and specific causes are listed. In addition, for each step of the metastatic pathway the effects of insensitivity to *anoikis*, as well as the key features, are listed.

amoeboid transition (MAT). MAT is typical of mesenchymal cells moving in non-stiff/rigid matrices, during selective inhibition of matrix proteases or integrin-mediated adhesions and has also been correlated with p53 or p27 loss of function mutations, all common events in progression of cancer towards malignancy [68,69]. Nevertheless both EMT and MAT are associated with malignancy and increase in metastatic colony formation, MAT undergoing cells do not enhance their resistance to *anoikis* [218]. Of note, MAT appears more correlated with the ability of cancer cells to cross endothelial barrier, irrespective to their ability to survive when suspended [218].

Anoikis resistance may also influence another key step of the metastatic process: the survival of cancer cells while they are circulating in the bloodstream. Indeed, after intravasation in the circulatory system, cancer cells totally lose any contact with solid tissues and should survive in a complete absence of ECM. Of course the development of pathways leading to *anoikis* resistance greatly facilitates the survival of cancer cells and their spreading to organs even very distant from the primary tumor site. In keeping with this idea it is noteworthy that circulating cancer cells, commonly found in several tumors and described as malignant cells, clearly show resistance to *anoikis* [16,110]. Of note, circulating cancer cells have been shown to bring their own soil with them when they circulate in the bloodstream. Indeed, cancer associated fibroblasts strictly associate with cancer cells, facilitating their transendothelial migration and accompanying cancer cells still remaining adherent to them and favoring their survival [61]. As cancer associated fibroblasts are able to elicit EMT in cancer cells, mainly acting through a NF-KB/HIF-1/Snail1 pathway [86,87], it is likely that the survival spur given by associated fibroblasts to circulating cancer cells is mainly due to the cross-talk between EMT and *anoikis* resistance pathways. In addition, it is also possible that cancer cells exploit the matrix proteins synthesized by their associated fibroblasts to engage pro-survival signaling. Future studies should reveal the reason for which cells bring with them fibroblasts and the identification of the molecular pathways should supply attracting pharmacological tools to fight dissemination of metastatic colonies.

An apparent contradiction of the association between EMT and metastasis comes from repeated observations that distant metastases derived from primary carcinomas are largely composed of cancer cells showing an epithelial phenotype, closely resembling that of the cancer cells in the primary tumor [173]. This discrepancy can be rationalized by the recognition that MET, the reversal of EMT, likely occurs following micrometastasis growing, due to local selective pressure for the outgrowth of cancer cells with more epithelial features or to the absence of EMT-inducing signals at sites of dissemination [173,223]. On the basis of the correlation between *anoikis* resistance and EMT one could argue that metastatic colonization and the consequent de novo achievement of an epithelial phenotype through MET may be associated with sensitivity to *anoikis* as well. Although data supporting this idea are still lacking, we should consider that the new organ in which cancer cells originate the metastatic colony is likely to contain an improper ECM for cancer cells, which should impede or decrease binding of the integrins expressed by cancer cells. In this view survival to improper adhesive stimuli is likely to be an important feature for cancer cells, irrespective by their possible MET. An intriguing idea, still to be investigated, is that cancer cells, once they are in the colonization site, first exploit their resistance to *anoikis* signaling and undergo MET only after their shift towards expression of a new set of integrins that correctly bind the ECM proteins of the new site (Fig. 7).

The final picture that we can draw illustrates *anoikis* resistance as a very useful feature for cancer cells, truly essential to obtain successful metastases. To date, several solid data sustain *anoikis* resistance as an attractive target in the fight against tumor progression, but honestly too many signaling pathways have been described to be efficiently targeted by therapy. A clearer identification of the mechanistic players in the cellular response, as well as their exact hierarchy, may be of high clinical significance in identifying successful approaches in fighting *anoikis* resistance, thereby impairing metastasis.

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