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Anoikis and EMT: Lethal “Liaisons” during Cancer Progression

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Abstract

Anoikis is a unique mode of apoptotic cell death that occurs consequentially to insufficient cell–matrix interactions. Resistance to anoikis is a critical contributor to tumor invasion and metastasis. The phenomenon is regulated by integrins, which upon engagement with components of the extracellular matrix (ECM) form adhesion complexes and the actin cytoskeleton drives the formation of cell protrusions used to adhere to ECM, directing cell migration. The epithelial-mesenchymal transition (EMT) confers stem cell properties and leads to acquisition of a migratory and invasive phenotype by causing adherens junction breakdown and circumventing anoikis in the tumor microenvironment. The investigation of drug discovery platforms for apoptosis-driven therapeutics identified several novel agents with antitumor action via reversing resistance to anoikis, inhibiting survival pathways and impacting the EMT landscape in human cancer. In this review, we discuss current evidence on the contribution of the anoikis phenomenon functionally linked to EMT to cancer metastasis and the therapeutic value of antitumor drugs that selectively reverse anoikis resistance and/or EMT to impair tumor progression toward the development/optimization of apoptosis-driven therapeutic targeting of metastatic disease.

Keywords

anoikis; epithelial mesenchymal transition; prostate tumor; integrin; talin

I. INTRODUCTION

Anoikis was originally defined as a unique mode of apoptotic cell death occurring upon insufficient cell-matrix interactions.^{1,2} The term is derived from the Greek “without a home” (*Anoikos*, becoming homeless) to describe the programmed death of epithelial and endothelial cells induced upon detachment from the extracellular matrix (ECM) consequent to insufficient cell-matrix interactions within the microenvironment. The ECM comprises a diverse network of cytokines impacting cell growth, motility, and angiogenesis, which can be made available for cellular use by enzymatic digestion and cytoskeletal remodeling.

The phenomenon of anoikis gained functional recognition and biological relevance in the cancer metastatic spread, as tumor epithelial cells upon release from the primary site and/or

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during dissemination in the circulation can undergo apoptosis and die prior to the metastatic journey. The development of resistance to anoikis and the mechanistic and phenotypic controls driving the process have been linked with tumor angiogenesis and metastasis^{1,3–5} because the mechanisms conferring resistance to this cell death enable the acquisition of migratory and invasive properties of tumor cells to establish metastatic lesions.⁶ The process of epithelial-mesenchymal transition (EMT) and consequential plasticity from cellular transdifferentiations due mesenchymal-epithelial transition (MET) critically contribute to tumor progression and to metastasis.⁶ The role of EMT in cancer takes central stage as tumor cells must physically detach from their primary tumor, invade the surrounding microenvironment, intravasate, endure the turbulence of circulation in the blood stream, and extravasate to secondary sites. The functional link between anoikis and EMT in dictating cancer cell survival outcomes has not been exploited.

Prostate cancer is a heterogeneous cancer with a natural history of progression from prostatic intraepithelial neoplasia to locally invasive androgen-dependent to androgen-independent metastatic disease, which is lethal.^{7,8} This malignancy is currently the second leading cause of cancer-related deaths among American men.⁹ Impairing the development of metastatic androgen-independent prostate tumors remains a major therapeutic endpoint for improving patient's survival.¹⁰ Androgen-independent prostate tumors develop resistance to standard treatment modalities (such antiandrogen therapy and radiotherapy) due to apoptosis and anoikis evasion and acquisition of invasive and metastatic properties navigated by EMT.^{5,10,11} Interrogating the mechanisms underlying treatment failure in patients with metastatic prostate tumors is the most critical therapeutic endpoint for improving patient survival.^{11,12} The emergence of castration-resistant prostate cancer (CRPC) is due to the activation of survival pathways, including apoptosis suppression, anoikis (apoptosis upon detachment from the ECM) resistance, aberrant androgen signaling, and increased neovascularization.^{6,13,14} The molecular exploitation of apoptotic signaling pathways has fueled the development of effective pharmacotherapies for the treatment of advanced metastatic CRPC.¹⁵ This review discusses the current understanding of mechanisms underlying anoikis resistance in metastatic cancer, as well as efforts to overcome such resistance and enhance therapeutic response in patients with CRPC.

II. SIGNALING DEATH TO “HOMELESS” CELLS

A. The Players

The intrinsic pathway of apoptotic death is triggered by intracellular signals that maintain cellular integrity and homeostasis, such as DNA damage and endoplasmic reticulum stress.¹⁶ Upon death-signaling activation, the cytoplasmic proapoptotic proteins Bax and Bak are translocated to the outer mitochondrial membrane (OMM). Subsequent oligomerization of Bax and Bak generates a channel within the OMM, contributing to mitochondrial permeabilization and cytochrome C release. Cytochrome C results in formation of the apoptosome, constituting caspase-9, cytochrome C and the cofactor apoptosis protease-activating factor (Apaf), which activates the effector caspase-3 and apoptosis.^{17–19}

The proapoptotic BH3-only proteins play a critical role during the intrinsic pathway in anoikis.²⁰ Bid and Bim, two members of this family, are activated by detachment of cells from ECM and facilitate the oligomerization of Bax-Bak within the OMM. The above members of the BH3-only protein family are indicated as activators.²¹ Specifically, sequestered Bim in the dynein cytoskeletal complexes is released until cells detach from the ECM and are subsequently translocated to the mitochondria.²² Loss of the cell-ECM interaction also leads to Bim accumulation, by inhibiting its proteasome degradation mediated by an ERK (extracellular signal-regulated kinases) and phosphatidylinositol-3-kinase and protein kinase B- (PI3K/Akt-) mediated phosphorylation of Bim, in the context of integrin engagement.^{22,23} Another group of BH3-only proteins are indicated as sensitizers, including Bad, Bik, Bmf, Noxa, and Puma. The sensitizer BH3-only proteins are incapable of promoting Bax and Bak oligomerization, but result in cell death by preventing activation of the antiapoptotic functions of Bcl-2.^{24–27} Indeed, Bcl-2 is the pivotal antiapoptotic family member which bypasses mitochondrial dysfunction and circumvents apoptosis by regulating Bak/Bax apoptotic members to prevent their clustering into pores, and by sequestering Bid and Bim activator members of the BH3-only proteins to prevent Bak/Bax oligomerization.^{27,28} Furthermore, other members of the BH3-only family are mechanistically involved in anoikis induction of different cell lines.^{29–31} For instance, Noxa and Puma, following transcriptional regulation by p53, have been shown to functionally contribute to fibroblast anoikis.^{29–31} In addition, in epithelial cells Bmf is capable of recording damage at the actin cytoskeleton and carrying on death signals towards cellular execution. Specifically, loss of the cell-ECM interaction leads to the release of Bmf from the myosin V motor complex with subsequent accumulation in the mitochondria and neutralization of Bcl-2 antiapoptotic action, inducing anoikis.^{30,31}

B. Extrinsic versus Intrinsic Forces of Death

The death receptor pathway is characteristically responsible for driving anoikis in both normal and malignant epithelial cells. Binding of the death ligands, including FasL or TRAIL, at the extracellular domain of the death receptor activates the pathway by causing oligomerization of the receptors.¹⁰ Ligand binding contributes to further binding of caspase-8 to the death effector domain (DED) of the Fas-associated death domain (FADD) and mediates the production of a death-inducing signaling complex (DISC). DISC further stimulates caspase-8 dimerization, which is cleaved to become active. The activated caspase-8 is released to the cytoplasm to activate downstream caspase-3 and -7, triggering apoptosis.^{10,32}

FLICE-inhibitory protein (FLIP) is the primary endogenous inhibitor of the death receptor pathway. This effector constitutes two DEDs and a C-terminal caspase-8-like domain with higher affinity to DISC. As such, FLIP is a more potent competitive inhibitor of procaspase-8, and thus blocks binding of caspase-8, preventing subsequent recruitments and activation.³³ In normal conditions, loss of cell contact with ECM contributes to upregulation of Fas and FasL and downregulation of FLIP expression. The above changes upon loss of cell-ECM interaction facilitate downstream caspase-8 activation in a FADD-dependent manner, leading to apoptosis.¹⁰ However, in malignant cells loss of the cell-ECM interaction fails to activate death receptor-induced caspase-8 despite the upregulation of Fas and FasL,

ultimately leading to anoikis resistance. Failure of cancer cells to downregulate FLIP after detachment may be responsible for the above effect.³² Studies have been performed that target FLIP in anoikis-resistant cells to generate a novel therapeutic approach to treating metastatic prostate cancer.³⁴ The involvement of caspases as an absolute requirement for cells in their “death row” has been challenged by the identification of caspase-independent pathways of apoptosis.³⁵ Bit1, a mitochondrial protein, translocates to the cytoplasm upon disruption of integrin-ECM interaction.³⁶ Through a cell death domain,³⁵ Bit1 induces apoptosis upon translocation to the cytoplasm in cells losing the integrin-ECM interaction.^{37,38} A decrease in Bit1 expression in the cytoplasm facilitates tumor progression in patients with invasive ductal carcinoma.³⁹ Mechanistically, cytoplasmic Bit1 facilitates cell death by reducing the activity of nucleus transducin-like enhancer of Split 1 (TLE1), a member of the Groucho/TLE/Grg protein family that mediates DNA-binding proteins to regulate transcription.^{40,41} Upon nuclear translocation, TLE1 heterooligomerizes with the amino-terminal enhancer of Split (AES), another member of the Groucho/TLE/Grg family.⁴² In cancer cells losing the integrin-ECM interaction, the AES/TLE1 heterooligomers are translocated from the nucleus to the cytoplasm following translocation of Bit1 into the cytoplasm.⁴³ The subsequent downregulation of nuclear TLE1 expression activates the death signaling pathway (Fig. 1).⁴⁴

C. Resistance to Anoikis Navigates Metastatic Journey

Cancer cells are capable of promoting anoikis resistance mainly through the regulation of integrins and the initiation of EMT. Integrins are essential for attachment of epithelial and endothelial cells to the ECM by recognizing fibronectin and laminin, the major adhesive ECM components.^{45,46} Integrin-mediated cell-ECM interactions are functionally involved in regulating tumor angiogenic response during cancer progression to metastatic disease.^{46–48} Integrins regulate signal transduction from the extracellular space to the intracellular network using integrin-activated signaling molecules, including the focal adhesion kinase (FAK), PI3-K, and members of the ERK1/2 mitogen-activated protein (MAP) kinase family.^{47–49} The loss of integrin-mediated epithelial cell-ECM interactions decreases phosphorylation of downstream effectors such as FAK, PI3-K, ERK1, and MAP kinase, thus mediating cell susceptibility to anoikis. The integrins β_1 , β_3 , and β_6 are upregulated during prostate cancer progression to metastatic disease.⁴⁸ Knockdown of β_1 integrin prevents anoikis resistance in PC3-MM2 cells, a highly metastatic cell line derived from PC3 cells passaged in mice.⁵⁰ Noteworthy, there is an association between increased β_1 integrin activation and consequential resistance to anoikis with prostate cancer progression and metastatic spread has been observed.⁵⁰

Mechanistically, taking over dynamic control of the focal-adhesion complex formation, β_1 -integrin mediates the activity of Src, the oncogenic Rous sarcoma virus.⁵¹ Cell adhesion functionally activates Src by ECM-integrin-mediated signals through FAK, while the loss of cell-ECM interaction leads to inactivation of Src, which contributes to anoikis.^{52,53} Activation of Src is associated with anoikis resistance in various cancers such as osteosarcoma, pancreatic carcinoma, and prostate cancer.^{52–55} Src is widely expressed in prostate cancer and has been studied as a potential therapeutic target to decrease prostate cancer metastases to the lymph nodes.⁵⁶

The integrin-linked kinase (ILK), a serine/threonine protein kinase, interacts with the cytoplasmic domain of β_1 - and β_3 -integrin, and is functionally involved in integrin and Wnt signaling pathways.^{57–59} In prostate cancer, ILK expression is associated with tumor progression and the increased proliferative index during progression; it is inversely correlated with 5-year patient survival.⁶⁰ ILK is essential in several integrin-involved cellular processes, including anchorage-dependent cell growth, cell adhesion, and fibronectin-ECM assembly.^{57,61,62} Cell-ECM adhesion contributes to ILK activation and directs phosphorylation of AKT Ser473 and glycogen synthase kinase-3 (GSK3); inhibition of ILK interrupts AKT phosphorylation and reduces cell survival.^{61,63} Interestingly, ILK-1 contributes to anoikis resistance by inhibiting apoptosis signals, such as initiator caspase-8 and executioner caspase-3, even in the absence of integrin/ECM signal activation, but possibly via a parvin-mediated targeting of AKT signaling to lipid rafts.^{64–66}

III. EMT LANDSCAPE IN CONTROL OF CELL FATE

EMT is a physiological process in which epithelial cells remodel cytoskeleton and cause the release of linkage with adjacent cells to generate a motile phenotype.⁶⁷ Reawakening this program during pathophysiologic conditions confers invasive and migratory properties to epithelia-derived cancer cells via coordinated molecular and genetic alterations. Dramatic phenotypic alterations transform an adherent epithelium into elongated, mesenchymal-like cells that are capable of evading normal tissue architectural constraints, allowing for their escape from the primary tumor mass. Mesenchymal-epithelial transition (MET) transdifferentiations have been implicated in the final stages of metastatic colonization to revert cells back to an epithelial-like phenotype upon settlement in distant tissues.⁶⁷ In addition, EMT allows cancer cells to detach from neighboring cells, to overcome anoikis, and to move from their primary location to metastatic sites.¹⁰ Cell polarity and epithelial integrity are maintained by tight junctions through claudins and occludins with one side attached to the ECM and adjacent cells.

A landscape of dramatic phenotypic alterations transforms an adherent epithelium into mesenchymal-like cells capable of evading normal-tissue architectural constraints, allowing their escape from the primary tumors.^{68–70} During EMT, cell-cell adhesion molecules, such as E-cadherin and γ -catenin, are downregulated, with a parallel upregulation of mesenchymal markers, such as α -smooth muscle actin (SMA), fibronectin, N-cadherin, and vimentin.⁶⁸ Acquisition of the mesenchymal phenotype is functionally associated with the ability to overcome anoikis.⁶⁹ Growing evidence links EMT induction to apoptosis evasion via upregulation of Bcl-2 antiapoptotic proteins and/or activation of the PI3-K/Akt prosurvival pathways, often associated with the downregulation of proapoptotic proteins such as Bim, Bax, Noxa, p21, and p53 effector related to pmp22Y.^{70–72}

The induction of phenotypic EMT is regulated by key transcription factors, including *Snail*, *Twist*, and *ZEB1/2*. Specifically, *Twist* activation contributes to EMT by facilitating migration and invasion, and upregulates the antiapoptotic protein Bcl-2 to promote anoikis resistance.^{73,74} Transcriptional activation of *Snail-1*, an E-cadherin repressor, is also involved in EMT induction.⁷⁵ In addition to E-cadherin, Snail can directly suppress the transcription of genes involved in anoikis, such as *PTEN*, downregulation of which activates

the PI3-K/Akt pathway and inactivates the proapoptotic protein Bad, thus contributing to anoikis resistance.⁷⁶ Moreover, downregulation of E-cadherin during EMT facilitates the accumulation of free β -catenin in the cytosol and subsequent nuclear translocation, which induces genes involved in the regulation of cell migration, invasion, and cell cycle progression, such as *c-Myc*, *cyclin D1*, *c-Jun*, and *MMP-1*.⁷⁷ Overexpression of β -catenin and its consistent presence in the cytosol leads to anoikis resistance via regulation of c-Myc, cyclin D1, and MAPK-mediated survival pathways and repression of epithelial genes toward sustaining a mesenchymal phenotype among cancer cell populations.⁷⁸ The ZEB1 transcription factor facilitates EMT^{79–81} via upregulation of vimentin and downregulation of E-cadherin and semaphorin 3F, resulting in the activation of Akt survival pathway and anoikis resistance.⁸¹

Therapeutic failure and subsequent disease progression to CRPC has been attributed to recurrent androgen receptor (AR) activity and the inability to completely suppress AR-mediated signaling.^{82–85} Rapidly growing evidence has defined diverse mechanisms responsible for AR signaling activation in metastatic CRPC, including AR amplification resulting in hypersensitivity⁸⁶; AR mutations and splice variants which reduce the ligand specificity required for receptor activation^{87–89}; alterations of AR coregulators⁹⁰; ligand-independent AR activation mediated by cross-talk with different signaling networks⁸³; and sustained androgen exposure or intratumoral androgen synthesis.⁹¹

Work in our laboratory suggests that modulating androgen exposure to androgen-independent prostate cancer cells contributes to EMT-MET cycling, actin cytoskeleton remodeling, and microtubule rearrangement.⁹² Furthermore, increasing androgen presence is associated with an augmented EMT phenotype in androgen-independent prostate cancer cells.⁹² Interestingly, the controversy surrounding the impact of androgen deprivation therapy (ADT) on the EMT landscape in normal mouse prostate tissue and preclinical models of LuCaP35 prostate tumor xenografts⁹³ is propagated in the clinical setting of human disease, where EMT signature profiles have been found in prostate tumors after ADT.^{94,95} Evidence from independent studies collectively supports elevation of EMT-related markers, including N-cadherin,⁹⁶ cadherin-11,^{97,98} and nestin,⁹⁹ in human prostate cancer specimens after ADT. Considering the transient nature of the EMT-MET cycling dynamic, the therapeutic window, relevant endpoints of treatment, and the EMT signature assessment might require coordination in capturing the temporal effect of androgen deprivation on prostate tumor epithelial cells prior to apoptosis induction among tumor subpopulations.

The canonical EMT signature driving the phenotypic process is programmed by a complex of gene expression changes. Epithelial markers include adherens and tight junction proteins (E-cadherin, ZO-1), and mesenchymal markers include the intermediate filament vimentin.^{100,101} Loss of cell polarity and gain of migration properties ensue. Genome-wide analysis of gene expression to identify primary targets of DZ-50 (an anticancer drug developed by our group¹⁴) identified the downregulation of two EMT effector genes, *Snail* and insulin growth factor binding protein 3 (*IGFBP3*), as well as *integrin- α_6* , *talin*, and *thrombospondin*, all of which regulate cell-ECM adhesion and angiogenesis; and claudin-11 and -14, which are transmembrane proteins involved in tight junction (TJ) strands formation.¹⁴ Selective targeting of claudin-11 during anoikis is significant as this TJ protein

interacts with the actin cytoskeleton.^{102–104} The focal adhesion effector, FAK, links AKT survival signaling to EMT via Snail activation.¹⁰⁵ IGFBP3, a secretory glycoprotein and IGF signaling regulator that promotes transforming growth factor beta– (TGFβ–) mediated EMT and confers antitumor resistance, is downregulated during anoikis induction by DZ-50.^{14,102} A large body of evidence supports the functional coupling of phenotypic EMT-MET cycling to anoikis signaling via diverse mechanisms involving the following: (1) FAK regulation of Snail induction via AKT survival signaling;¹⁰⁵ (2) coordinated growth factor signaling network suppression of anoikis via proapoptotic proteins;¹⁰⁶ (3) recruitment of FAK and paxillin to β₁ integrin–promoting cancer cell migration via MAPK activation;¹⁰⁷ (4) combined activation of FAK and AKT survival signaling¹⁰¹; and (5) regulators of fibronectin-ECM assembly through EMT transcription factors that dictate anoikis outcomes.^{57,64,106,108,109}

IV. REMODELING THE ACTIN CYTOSKELETON

The actin cytoskeleton is an essential scaffold for integrating membrane and intracellular functions. Growth stimulation promotes the actin polymerization assembly to generate movement, whereas apoptosis elicits cytoskeletal reorganization changes.^{110,111} The regulation of anoikis outcomes in epithelial cells by the actin cytoskeleton is supported by compelling data on the involvement of key regulators of cytoskeleton organization in cell motility. Thus, the functional loss of cofilin, the main actin regulator, significantly decreases prostate cancer cell adhesion¹¹² while the mutation at the S3A of cofilin compromises cell migration and filopodia formation due to enhanced F-actin filament polymerization.¹¹² Another effector, Ankyrin G, has been shown to functionally link EMT to the cytoskeleton via its localization to AJ and interaction with E-cadherin.¹¹³ Moreover, overexpression of LIM kinase-2 (LIMK2), which regulates cofilin phosphorylation and actin dynamics, is associated with invasive prostate cancer¹¹⁴; and claudin-11, a TJ transmembrane protein that interacts with the actin cytoskeleton, is targeted by the anoikis-inducing agent DZ-50.^{14,102} Taken together, these data support the targeting of interactions between actin regulators and tight junctions responsible for resistance to anoikis to overcome therapeutic failure.

Talin1 is an actin-binding protein that links integrins to the actin cytoskeleton in focal adhesion complexes.¹¹⁵ The integrity of ECM-fibronectin sustains high talin levels, thus conferring resistance to DZ-50–induced anoikis, evidence that can be of therapeutic significance in targeting of talin in advanced prostate tumors to lift resistance to anoikis.¹⁴ This gains indirect support from clinical evidence on the correlation between elevated talin1 and human prostate cancer progression to metastasis.⁵ The possibility of simultaneous induction of both apoptosis and anoikis, effectively enhancing the therapeutic response to antitumor agents by massive cell killing, gains support from clinical evidence in human breast cancer. During tumor progression, promotion of metastasis is exerted by Bcl-x_L, a suppressor of cytoskeleton-dependent apoptosis and enhancer of metastasis but not primary tumor growth.¹¹⁰

In a complex signaling repertoire, talin1 binding to β integrin recruits the focal adhesion partners ILK, FAK, and Src, and activates the downstream signals PI3-K/AKT and ERK, preventing anoikis and promoting cell invasion.^{14,116,117} Specifically, talin1 S425

phosphorylation, essential in β_1 integrin activation, correlates with metastatic properties of prostate cancer cells. Nonphosphorylatable talin1 (S425A) expression in talin1-silenced PC3-MM2 and C4-2B4 prostate cancer cells leads to integrin-mediated adhesion, motility, and increased susceptibility of cells to anoikis, all of which are consequential effects of decreased activation of β_1 integrins.¹¹⁸ Work in our laboratory has established that talin's contribution to anoikis resistance can be overcome by DZ-50, with potential therapeutic targeting value in metastatic prostate cancer.^{5,10,14} The mechanistic dissection of DZ-50 gene targets in prostate cancer cells reveals key receptor kinases, including LIMK2, GSK3 β , AKT, and MAP4K5, that are involved in anoikis regulation. LIM kinases regulate cofilin phosphorylation and actin cytoskeletal dynamics by phosphorylating its Ser3 residue and inactivating its depolymerization activity.⁸ Actin polymerization regulated by cofilin activation is a convergence point in intracellular signaling through which extracellular stimuli impact the actin cytoskeleton, cell invasion, and apoptosis.^{119–121} In a signaling cascade via a TGF- β -directed mechanism, constitutively active cofilin (S3A mutation) promotes filopodia formation and cell invasion and directs the EMT landscape in prostate cancer cells.¹¹² In clinical specimens of human prostate cancer, there is significant overexpression of cofilin in metastatic compared to primary prostate tumors.¹¹² Considering that the phosphorylation status/activity of cofilin can dictate interactions of the actin cytoskeleton with ECM effectors, enabling acquisition of metastatic properties,⁵⁹ targeting of cofilin can lift anoikis resistance in metastatic cells with potential therapeutic significance.

V. THERAPEUTIC SIGNIFICANCE OF OVERCOMING ANOIKIS RESISTANCE

A. Quinazoline Derivatives

Our own drug discovery efforts focusing on the structural optimization of a quinazoline-based α_1 -adrenoceptor antagonist, Doxazosin, led to the generation of a lead derivative, DZ-50. DZ-50 impaired prostate tumor growth and metastasis via anoikis induction and antiangiogenesis in prostate cancer cell lines and human prostate cancer xenografts in nude mice.^{14,122} The results of a genome-wide microarray analysis in human prostate cancer DU-145 cells to identify the primary molecular targets of DZ-50 indicates that DZ-50 downregulates genes encoding regulators of ECM (*fibronectin* and *integrin- α_6*), tight junctions (*claudin-11*), angiogenesis (*thrombospondin*), and the critical effector of the IGF axis, *IGFBP3*. IGFBP3 induction is essential for prostate stroma remodeling representing an attractive therapeutic target by the novel quinazoline DZ-50.^{14,123}

B. Src Inhibitors

The Src family of kinases has recently been evaluated as a potential therapeutic target to develop treatment for a variety of cancers. Dasatinib, a Src family tyrosine kinase inhibitor, at low concentrations, represses prostate cancer cell adhesion, migration, and invasion.¹²⁴ Dasatinib effectively suppresses both tumor growth and development of lymph node metastases in both androgen-sensitive and androgen-resistant prostate tumors in preclinical models.⁵⁶ Significantly enough, an anoikis-resistant and spheroidogenic intermediate mesenchymal state phenotype of SKOV3 ovarian cancer cells was reversed by the Src kinase inhibitor AZD0530 via increasing E-cadherin promoter activity and E-cadherin expression

levels in both in vitro and in vivo preclinical studies.¹²⁵ The Src inhibitor, Dasatinib, clinically approved for patients with chronic myelogenous leukemia, demonstrated a limited therapeutic response in patients with metastatic CRPC.¹²⁶ A single patient, however, exhibited a significant therapeutic response, indirectly supporting further molecular profiling to target selective patient populations.¹²⁶ The clinical evidence, in terms of the combination of such an anoikis-inducing agent with Docetaxel chemotherapy, failed to provide an additional therapeutic benefit in metastatic CRPC.¹²⁷ Bosutinib, another Src inhibitor, in combination with ABT-263, a Bcl-2 inhibitor, effectively induces anoikis in lung adenocarcinoma and in advanced leukemia resistant to kinase inhibitors.^{128,129}

C. Trks Inhibitors

Tropomyosin-related kinases (Trks), a family of receptor tyrosine kinases (RTK) activated by neurotrophins, are involved in tumor cell growth and survival signaling.¹³⁰ The pan-Trk inhibitors have been evaluated in tumor xenograft and transplantation models using neuroblastoma, medulloblastoma, and prostatic and pancreatic cancer cell lines.¹⁰ Furthermore, a Trk inhibitor, K252a, attenuates brain-derived neurotrophic factor-induced migration and proliferation of nasopharyngeal carcinoma cells by exerting potent anoikis sensitization.¹³⁰ Trk inhibitors can be used as anoikis-sensitizing agents in diverse human malignancies with a promise of overcoming therapeutic resistance and stopping lethal disease. MGCD516, an RTK inhibitor with Trk inhibitory activity, is being evaluated in a Phase I clinical trial in patients with clear cell renal cell carcinoma resistant to angiogenesis inhibitors and in CRPC patients with advanced bone metastasis ([Clinicaltrials.gov](https://clinicaltrials.gov)).

D. c-Met/VEGFR-2 Inhibitors

c-Met and VEGFR-2 (vascular endothelial growth factor receptor-2), both functionally critical players in cancer initiation and progression to metastasis,¹³¹ can be therapeutically targeted by exploitation of anoikis. Indeed, inhibition of c-Met and VEGFR-2 with foretinib reduces ovarian cancer cell adhesion, migration, and invasion via anoikis induction, impairing ovarian tumor growth.¹³² Considering the evidence discussed so far, one may easily argue that anoikis potentially drives the clinical antitumor action of foretinib in advanced renal cell and gastrointestinal carcinoma.¹³²

E. Metformin

Of direct clinical significance is recent evidence that the antidiabetic agent metformin exhibits anoikis resistance-lifting properties in preclinical models of human cancer. In medullary thyroid cancer (MTC), it inhibits mammalian target of rapamycin (mTOR)/6SK and pERK survival signaling,¹³³ and sensitizes MTC cells to anoikis.¹³³ In clinical trials with prostate cancer patients, metformin significantly reduces biochemical recurrence¹³⁴ and stabilizes disease in chemotherapy-naïve CRPC patients,¹³⁵ implicating anoikis-mediated therapeutic benefits in patients post-ADT.¹³⁶

VI. SUMMARY AND FUTURE DIRECTIONS

Both anoikis resistance and EMT reversal to MET have been implicated in the development, progression, and therapeutic failure of different tumors. Considering the transient nature that

characterizes both phenomena—anoikis and EMT-MET cycling—targeting these phenotypic processes during a defined therapeutic window during mesenchymal transition—immediately upon disruption of the tumor epithelial cell-ECM interaction and prior to initiation of metastatic dissemination—calls for personalized treatment of cancer patients. Coordinated activation of apoptosis and anoikis signaling mechanisms allow for the combination targeting of primary tumor growth and impairing metastatic progression, depending on the EMT landscape of individual tumors.

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ABBREVIATIONS

AR	androgen receptor
DED	death effector domain
DISC	death-inducing signaling complex
ECM	extracellular matrix
EGFR	epidermal growth factor receptor
EMT	epithelial-mesenchymal transition
ERK	extracellular signalregulated kinase
FADD	Fas-associated death domain
FAK	focal adhesion kinase
FLIP	FLICE inhibitory protein
GSK-3	glycogen synthase kinase-3
ILK	integrin-linked kinase
MAPK	mitogen-activated protein kinase
MET	mesenchymal-epithelial transition
MMP	metalloproteinase
OMM	outer mitochondrial membrane
PI3K	phosphoinositide-3-OH kinase
PPAR-γ	peroxisome proliferator-activated receptor-gamma
PTEN	phosphatase and tensin homolog deleted on chromosome 10
RTKs	receptor tyrosine kinases

SMA	α -smooth muscle actin
TGFβ	transforming growth factor beta
Trks	tropomyosin-related kinases
VEGFR	vascular endothelial growth factor receptor

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FIG. 1.

Anoikis signaling pathway in cancer cells. Cell detachment-induced anoikis signaling can be activated via three pathways: (1) the extrinsic death receptor-mediated apoptotic pathway, (2) the intrinsic apoptotic pathway, and (3) the caspase-independent pathway. In the first pathway, activation of Fas/FADD leads to cleavage of caspase-8 and subsequently activates downstream caspase-3 to facilitate apoptosis. In the second pathway, Cell-ECM interaction mediates integrin-induced activation of Talin1, FAK/Src and ILK-1. ILK-1 activation leads to phosphorylation of GSK3 β and subsequent recruitment of Snail, which ultimately activates the PI3-K/Akt cell survival pathway. The loss of cell-ECM interaction contributes to upregulated proapoptotic proteins, such as Bim and downregulated antiapoptotic regulatory proteins, such as Bcl-2. The above changes activate cytochrome C release from the mitochondria, which subsequently activates caspase-9 and -3 to induce apoptosis. In the third pathway, upon cancer cell loss of the ECM interaction, the AES/TLE1 heterooligomers are translocated from the nucleus to the cytoplasm; subsequent downregulation of TLE1 by the proteasome results in the activation of the death signaling pathway.