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NON-THEMATIC REVIEW

Integration of EMT and cellular survival instincts in reprogramming of programmed cell death to anastasis

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Abstract
Apoptosis is a tightly controlled, coordinated cellular event responsible for inducing programmed cell death to rid the body of defective or unfit cells. Inhibition of apoptosis is, therefore, an essential process for cancer cells to harness. Genomic variants in apoptotic-controlling genes are highly prevalent in cancer and have been identified to induce pro-proliferation and pro-survival pathways, rendering cancer cells resistant to apoptosis. Traditional understanding of apoptosis defines it as an irreversible process; however, growing evidence suggests that apoptosis is a reversible process from which cells can escape, even after the activation of its most committed stages. The mechanism invoked to reverse apoptosis has been termed anastasis and poses challenges for the development and utilization of chemotherapeutic agents. Anastasis has also been identified as a mechanism by which cells can recover from apoptotic lesions and revert back to its previous functioning state. In this review, we intend to focus the attention of the reader on the comprehensive role of survival, metastasis, and epithelial mesenchymal transition (EMT), as well as DNA damage repair mechanisms in promoting anastasis. Additionally, we will emphasize the mechanistic consequences of anastasis on drug resistance and recent rational therapeutic approaches designed to combat this resistance.

Keywords Anastasis · EMT · DNA repair · Cell survival · Drug resistance

1 Introduction

Cancer is the second leading cause of death globally. A recent report by the World Health Organization suggests that cancer is responsible for approximately 9.6 million deaths worldwide in 2018 alone [1]. Chemotherapy has remained a cornerstone in the treatment of cancer for decades and mechanistically relies on the activation of apoptosis to exert its effect [2]. Development of tools and techniques in physiology and medicine has adequately improved the modus operandi of new-generation drugs and hence facilitating cancer care; still, cancer-related deaths continue to increase at an alarming rate [3].

Due to the reliance of traditional/modern chemotherapeutic agents on triggering apoptosis for their efficacy, understanding of the molecular complexity of apoptotic processes is vital to the development and utilization of chemotherapeutic drugs. A major challenge for the efficacy of chemotherapy is failure in attaining apoptotic plateau among heterogenic population of cancer cells. That is to say that chemotherapeutic agents must achieve a certain level of apoptotic signaling to ensure entry into programmed cell death for a cancer cell. Hence, failure to induce apoptosis throughout sub-clonal populations within a tumor allows cancer cells to escape programmed cell death by activating multiple survival strategies. Such a process has recently been described by several authors wherein cancer cells undergoing programmed cell death could revert to a normal physiological state once pro-apoptotic agents, including

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chemotherapy, are removed from the system. This process is termed anastasis, from the Greek word meaning rising to life, juxtaposed to apoptosis, derived from the Greek meaning falling to death [4–6]. Recent research in the field of anastasis has unveiled several cellular signaling factors that play a vital role in cell death recovery and are highly linked with cancer cell survival and metastasis [7, 8]. Hence, in this review, we will discuss the potential role of anti-apoptotic factors and signaling related to EMT/metastasis in aiding cancer cells to maintain survival state and their mechanistic links to anastasis. Additionally, we will emphasize the consequences of anastatic mechanisms in cancer drug resistance and explore alternative therapeutic approaches to minimize the hindrances caused by these mechanisms to attain cell death to optimize chemotherapeutic agents.

2 Broad perspectives of pro-proliferative signaling in facilitation of cancer survival over apoptosis

Apoptosis is a well-controlled and coordinated process within the cell that is designed in such a manner that anti-apoptotic/pro-survival proteins can halt or revert its progression, hence facilitating cells to resist cellular stress [9, 10]. While this balance of pro-death/pro-survival signaling is important in sustaining cellular homeostasis, cancer cells take advantage of this fail-safe mechanism to maintain survivability and hyperactivated oncogenic responses [11]. Chemotherapeutic drugs designed to induce apoptosis must overcome this pro-survival signal which, if not overcome, eventually aids in cancer drug resistance. Carfilzomib, a proteasome inhibitor that induces apoptosis, has been studied in the context of pro-survival mediated apoptotic resistance as discussed above. Recent studies in k-Ras mutated colorectal carcinoma suggest that the expression of BCL-XL, a major survival protein of BCL-2 family, is elevated and is responsible for carfilzomib-mediated resistance and is reversed upon knockdown or pharmacologic inhibition of BCL-XL by sh-RNA or by using BCL-XL antagonist ABT-263 [12]. Similarly, simultaneous inhibition of pro-survival genes (*FLIP*, *BCL-XL*, *MCL1*, *SURVIVIN*, *XIAP*, and *K-RAS*) concerning apoptotic checkpoints by using si-RNAs has been shown to trigger robust apoptosis in cancer cells compared with silencing individual genes, hence underscoring the synergistic effects of survival factors in impeding apoptotic mechanisms (Fig. 1) [13].

Ultimately, cellular fate depends upon the balance between pro-apoptotic and pro-survival pathways [14]. This equilibrium is best explained in preclinical settings where subtoxic cellular stressors are introduced *in vitro* to measure changes in cellular signaling pathways [14–16]. For example, a sublethal concentration of camptothecin, a topoisomerase inhibitor, has been demonstrated to induce co-expression of pro-survival factors, *viz* NFκB, c-FLIP, survivin, and p-AKT, along with pro-apoptotic proteins such as BAX and BID. This pro-survival action induced by low-dose camptothecin is joined by simultaneous binding of annexin-V to flipped phosphatidylserine as well as early disruption of the

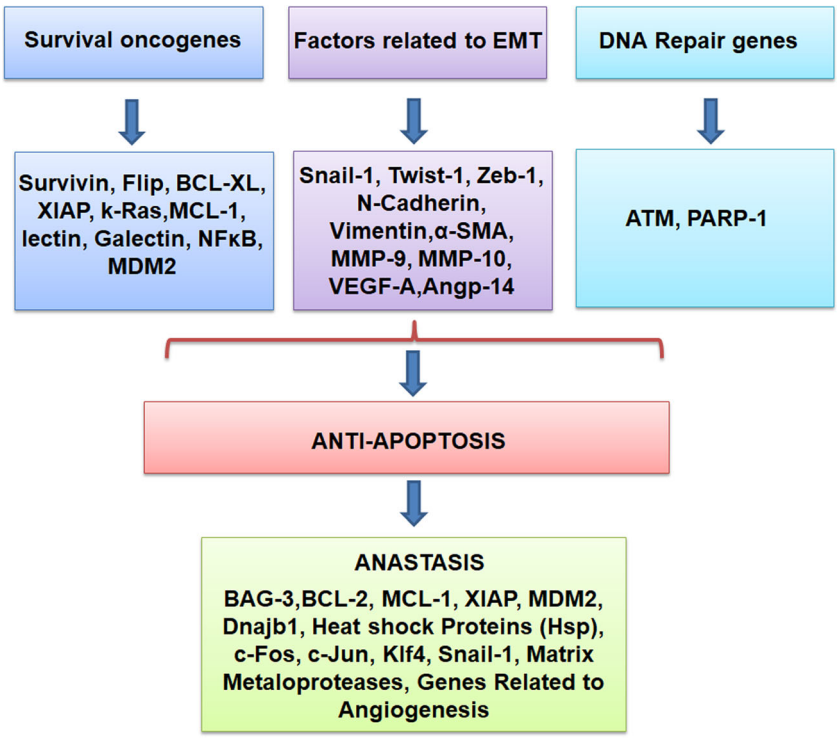

Fig. 1 Mechanisms involved in anastasis encompassing cellular survival, EMT, and DNA repair. Genes associated with these pathways form the basic framework to execute the process of anastasis

mitochondrial membrane [17]. This demonstrates the delicate and simultaneous activation of both pro-survival and pro-apoptotic processes active during cellular stress, and the critical balance between their respective mediators eventually determines the cellular fate. A similar preconditioning effect is observed in lung and hepatocellular carcinomas, where subtoxic doses of aclarubicin, an anthracycline agent, upon pretreatment or in combination with doxorubicin, reduced the cytotoxicity and DNA damaging potential of doxorubicin by inducing pro-survival signals [18]. Likewise, in preclinical models of de-differentiated liposarcoma, low doses of doxorubicin amplify MDM2 expression resulting in survival benefits to these cells through inhibition of p53-induced apoptosis and resistance to doxorubicin-mediated cytotoxicity [19]. These reports suggest that cellular stress does not result in a continuous correlation with induction of apoptosis; rather, a dichotomous relationship exists where pro-apoptosis signaling must overcome a certain plateau in order to activate apoptosis completely. Treatments with cytotoxic agents that fail to reach this apoptotic plateau induce drug resistance through the activation of pro-survival pathways.

Hyperactivated pro-survival/oncogenic proteins (tyrosine kinases, k-Ras, MAPK, AKT, PAK1, *etc.*) not only trigger anti-apoptotic signaling but also facilitate cellular disintegration by activating EMT by a non-canonical route [20]. From genesis itself, cellular migration and survival mechanisms are deeply connected, sharing common transcription factors (NFκB, STAT3, HIF1α, *etc.*). This connection makes tumor dissemination an inevitable process in cancer while also being highly linked with chemotherapeutic resistance [21–26]. While anti-apoptotic factors like MDM2, BCL-2, survivin, and c-FLIP have been reported to be intricately linked with pro-metastatic mechanisms [27–30], pro-apoptotic proteins like Bid, Bax, and Par-4 are inversely co-related with pro-invasive signaling (Fig. 1) [28, 31]. Hence, the survival mechanisms adopted by a growing tumor to curb its internal stresses, including hypoxia, immunogenic, oxidative, and unfolded protein response stressors, may also be a driving force for cancer metastasis. Whether metastasis-promoting factors also play a role in cellular survival and anti-apoptotic signaling is the subject of ongoing study.

3 Epithelial-mesenchymal transition as a mechanism to deceive cell death

3.1 Transcription factors regulating EMT simultaneously facilitate cancer cell survival

A unique characteristic in malignant transformation of cells is their ability to migrate and propagate tumors at secondary sites. The processes involved in cellular migration are highly complex and engross both molecular and structural changes, thus making cancer cells highly dynamic [32]. The transition of malignant epithelial cells to mesenchymal morphology, termed epithelial-mesenchymal transition (EMT), is believed to be one of the most prominent structural changes that takes place in primary metastasized cells and known to be activated by transcription factors including Snail-1/2, helix loop helix TF Twist-1, and Zeb-1 [33]. Transcription factors and modulators of EMT apart from its role in cellular migration are also suggested to facilitate survival mechanisms through hindering apoptosis (Fig. 1) [34, 35]. Studies conducted in canine MDCK and mouse embryos elucidate that Snail-1 expression triggers resistance to cell death. In these studies, MDCK cells stably expressing the Snail-1 transcription factor exhibited cell cycle arrest and resistance to cell death induced by serum depletion and TNF-α treatment, primarily mediated by the simultaneous activation of PI3K and MAPK pathways. Similarly, in developing mouse embryos, elevated Snail-1 expressing regions of the premigratory neural crest and primitive streak shows resistance to cell death and cell cycle arrest [36]. Correspondingly, Slug, a Snail family transcription factor, disrupts BH3-mediated inactivation of major survival factor BCL-XL, thus barring intrinsic apoptosis [37]. Apart from Snail and Slug, other transcription factors of EMT are also connected to anti-apoptosis signaling. Contextually, Maestro et al. elucidated that survival fractions within Myc induced apoptotic population of cells activate pro-survival factors of the BCL-2 family while selective enrichment of BCL-2 expressing survival populations demonstrates elevated expression of Twist-1. The result of this analysis suggests that Twist-1, an essential helix loop helix transcription factor, exerts an oncogenic role by hindering apoptosis [38]. Zeb-1, an essential EMT transcription factor, is expressed in the non-invasive neoplastic stage of pancreatic adenocarcinoma both in clinical and animal models, aiding in tumor proliferation and maturation [39]. Recent reports also suggest that Zeb-1 disrupts the oncosuppressive role of p53 and its associated retinoblastoma protein, thus impeding p53-mediated senescence and apoptosis (Fig. 2) [40].

A majority of the upstream regulators of EMT are linked with cancer cell proliferation and survival; however, TGF-β, a critical mediator of EMT-related transcription factors, is known to activate apoptosis [41]. This complexity of TGF-β signaling in EMT was recently addressed from a study conducted by David et al. in pancreatic ductal adenocarcinoma murine model. In TGF-β sensitive cells, Smad complexes (Smad 2/3/4) were identified to transcribe Snail-1 and Sox-4 genes. The Smad2/3/4 complex subsequently interacts with Snail-1 to repress the transcription of KIf5, resulting in Sox-4-driven switching from EMT to apoptosis. Intriguingly, Smad-4 depletion confers TGF-β-mediated EMT signaling tumorigenic since the Smad 2/3 complex transcribes only Sox-4, which in combination with KIf5 promotes malignancy (Fig. 2) [42]. Moreover, Smad-4 mutation is widespread

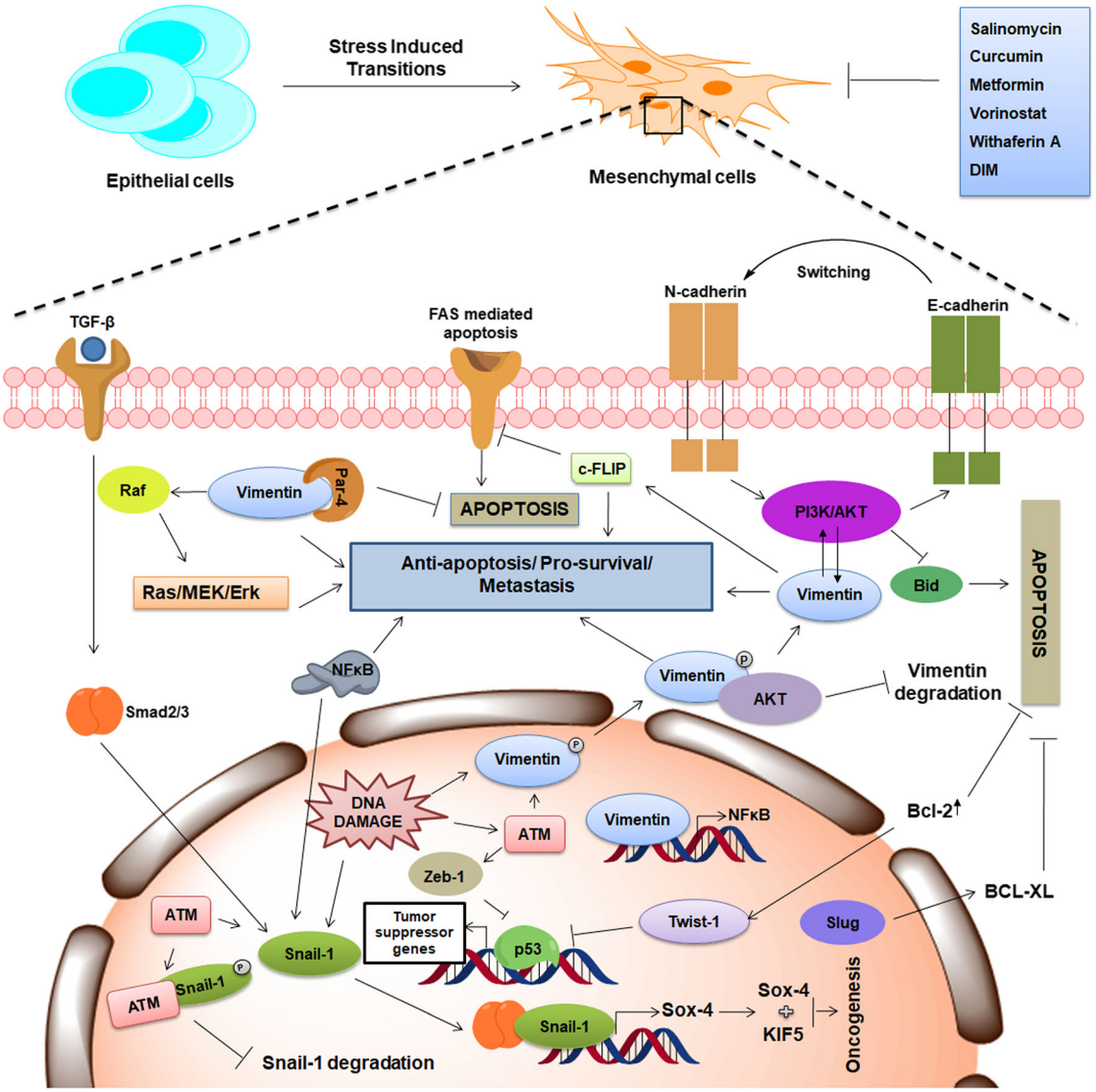

Fig. 2 Signaling network describing the activation of EMT-related proteins, including Snail-1, Twist-1, and vimentin, by DNA damage response factor ATM kinase. The activated Snail-1 modulates cellular survival elements, *viz* PI3K and MAPK. Twist-1, however, blocks the transcriptional role of p53 that in turn is activated by BCL-2 upregulation. Vimentin, a major EMT modulator, is activated *via* ATM kinase and further aids in transcribing NFκB. Vimentin also regulates the activity of c-Flip, thus controlling FAS trafficking. TGF-β-mediated activation of Smad 2/3 complex further facilitates Snail-1. In the absence of Smad-4, Snail-1 interacts with Smad 2/3 complex and binds to the Sox-4 promoter, thus stimulating carcinogenesis. DNA damage sensor proteins can amplify AKT signaling and thus prevent vimentin degradation by interacting with its phosphorylated form

across many cancers [43]; therefore, by canonical or non-canonical routes, EMT activation in most instances is linked with pro-tumorigenic and anti-apoptotic signaling.

To cut a long story short, cancer metastasis is an adaptive process, where cells following EMT activation device certain strategies (avoiding anoikis and immunogenic cell death) to deceive cell death for its successful migration [44, 45]. Therefore, cellular survival mechanisms are an integral part of EMT signaling involving not only the EMT transcription factors but other modulators too. For example, N-cadherin plays a crucial role in cellular survival by activating the PI3K/AKT pathway, which eventually dampens the action of the pro-apoptotic protein Bid. This cascade further aids in E-cadherin to N-cadherin switching [46, 47]. Likewise, vimentin, a vital type-3 intermediate filament having a cytoskeletal and migratory role during EMT, demonstrates a pro-tumorigenic role by preventing caspase-3-mediated AKT-1 proteolysis through its direct interaction with the vital pro-survival kinase [48]. Vimentin is also reported to interact with the 14-3-3 protein, hence blocking the sequestration of Raf kinase and maintaining Ras/Raf/Erk pro-proliferative signaling [49, 50]. The anti-apoptotic function of vimentin is so extensive that it also physically interacts with Par-4, a major pro-apoptotic protein, to disrupt its apoptotic activities (Fig. 2)

[51]. Other structural effectors of EMT, like matrix metalloproteases, α-SMA, and fibronectin, are also reported to activate pro-survival responses within migrating cancer cells [52–54]. Collectively, these evidences suggest that pro-survival signaling and EMT are highly connected, resulting in suppression of apoptosis and facilitation of both tumor migration and progression (Fig. 1).

3.2 Drug-induced EMT is associated with chemoresistance

Traditional cytotoxic chemotherapeutic agents have been identified to induce resistance across different cancer types, in which EMT has also been identified to play a critical role in hampering drug sensitivity [55–58]. In relation to this concept, our group has recently conceived a novel approach wherein Chakraborty et al. establish that during the initial phase of treatment with DNA-damaging drugs, cancer cells of epithelial origin begin expressing mesenchymal markers. As discussed in the previous section, EMT is highly connected with survival factors. In this study, drug-induced EMT is also associated with pronounced activation of survival factors, *viz* NFκB, c-FLIP, survivin, and AKT, respectively. Interestingly, signatures of early apoptosis are also co-parallelly accompanying EMT/cell survival proteins. Moreover, in this study, the EMT modulator vimentin hinders the ongoing apoptotic progression and delays late-stage apoptotic features such as caspase-3 activation and DNA damage response (Fig. 3) [17]. Similarly, Fischer et al. described that EMT is more prevalent in drug-induced metastasis compared with spontaneous metastasis. The authors created an autochthonous breast cancer murine model wherein transgenic mice bearing a Cre recombinase construct flanked by Fsp-1 (fibroblast-specific protein 1) promoter would expresses Cre recombinase when the Fsp-1 promoter is activated; specifically, in mesenchymal lineage as this protein is expressed explicitly in cells undergoing EMT. Once the Cre recombinase is transcribed, it then knocks out a red fluorescent protein gene (RFP) of another transgenic construct including GFP (green fluorescent protein). This complex combination of constructs was designed in such a manner that when the RFP gene was knocked out, cells would express only GFP, thus indicating activation of EMT. This model convincingly demonstrates that during spontaneous metastasis to the lung, few cells expressed GFP; however, mice treated with cyclophosphamide demonstrated significant expression of GFP in metastatic lung nodules. GFP-positive cells harvested from metastatic sites were also found to be highly resistant to chemotherapy as opposed to GFP negative cells [59].

4 Cell death recovery by anastasis—a process opposing apoptosis

4.1 Apoptosis: evolving views

Apoptosis is an evolutionary conserved phenomenon in eukaryotes responsible for maintaining cellular homeostasis by eliminating defective and potentially harmful cells [9]. Moreover, stresses (unfolded protein response, oxidative stress) within the cell that disrupt normal homeostasis as well as external signaling cues such as immune modulators activate mitochondrial and receptor-mediated apoptosis [60]. Once activated either *de novo* or extrinsically, the entire process culminates in

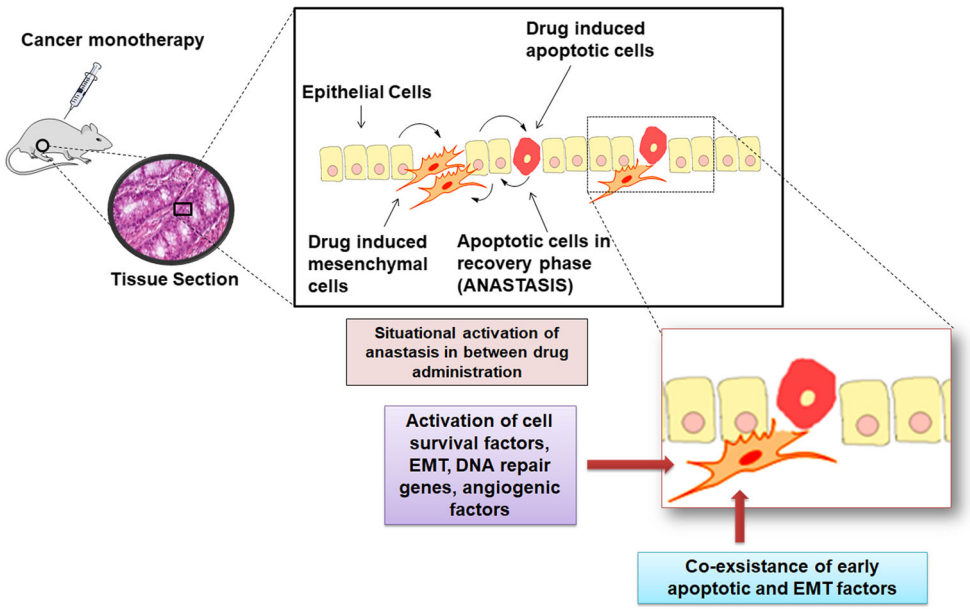

Fig. 3 Overall schematic representing the development of drug-induced EMT and activation of anastatic processes *in vivo*. Cancer monotherapy often triggers EMT in cancer cells. These signals also mediate apoptotic and drug resistance. Moreover, EMT-activated cells also demonstrate signatures of early apoptosis

caspase activation leading to its cleavage and further triggering of molecular events including membrane blebbing, cell shrinkage, nuclear fragmentation, and chromatin condensation. Traditionally, these processes are thought to be irreversible in nature, thus making apoptosis the most vital programmed cell death mechanism in the cell [61]. A majority of chemotherapeutic drugs are designed to facilitate this process in cancer cells; however, cancer cells, due to their overwhelming survival instincts [62], tend to block the processes of apoptosis and continue to proliferate at an exponential rate [14].

Our understanding of apoptosis is gradually evolving, and upcoming reports challenge the traditionally understood irreversibility concept of programmed cell death. Hence, the cancer cells undergoing programmed cell death can now revert to a normal physiological state once pro-apoptotic stressors, including chemotherapy, are removed from the system (Fig. 4) [63]. This process is referred to as anastasis, as described before. Recovery from the brink of early apoptosis was described primarily by Hammill et al. wherein B cell lymphoma (BCL) cells recover from early stages of apoptosis upon removal of an immunotherapeutic agent (anti-Ig antibody) [64]. Similarly, Geske et al. also describe recovery of cancer cells from early stages of apoptosis wherein the authors induced p53-mediated apoptosis in p53-truncated mouse mammary carcinoma cells by transfecting with temperature-sensitive p53 construct. In non-permissive temperature, the expression of p53 gets halted and the authors observe reversal of early stages of apoptosis [65]. Likewise, human cancer cells HeLa, A357, HepG2, PC3, and MCF-7 have been shown to recuperate from late stages of apoptosis when the chemotherapeutic agent jasplakinolide is removed from the system. Surprisingly, critical signatures of apoptosis such as cell shrinkage and membrane blebbing, which were previously thought to be irreversible, successfully revert within 14 h of apoptotic agent removal [5].

This budding concept of anastasis has been documented in many organisms, including both invertebrates and vertebrates [63]. In a recent study utilizing a drosophila model, cells with induced caspase activation are observed to sustain healthy conditions for prolonged periods, gaining resistance to late-stage apoptosis [66]. Similarly, caspase activation during the differentiation process in the lens of mammals has been found

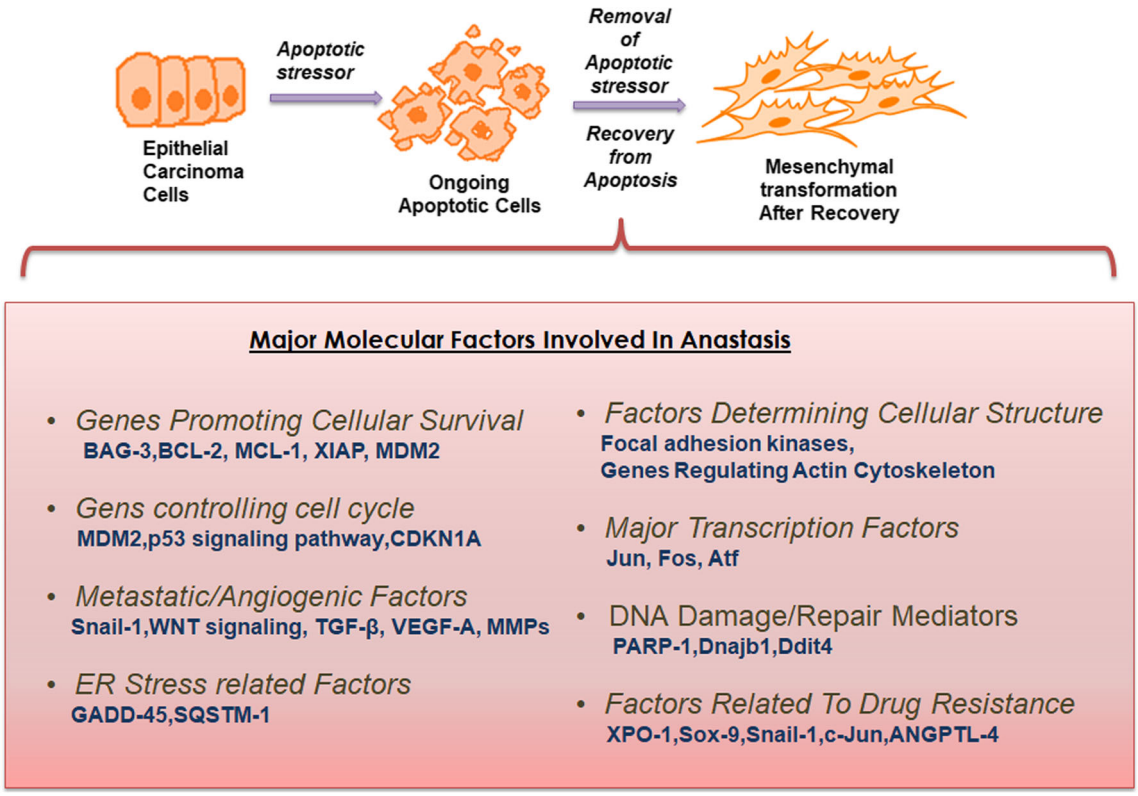

Fig. 4 Schematic representation of the structural and molecular changes during anastasis. Cancer cells demonstrating the signatures of apoptosis upon treatment with apoptotic agent. These cells recover completely from apoptosis when the stress-inducing agent is removed by activating anastasis. Moreover, cells recovering from apoptosis show mesenchymal features and plethora of molecular changes which include induction of genes promoting cellular survival, metastasis, and angiogenesis. Factors related to protein processing, cell cycle control, and DNA repair are also expressed. Cells that recover from death by activating anastasis displayed pronounced structural changes by altering focal adhesion kinases and activating genes related to actin cytoskeleton. Apart from mesenchymal features, proteins associated with chemoresistance are also elevated in cells undergoing recovery from cell death

to promote DNA fragmentation and PARP cleavage, reiterating the hallmarks of apoptosis. However, this caspase activation was proven non-lethal and an essential prerequisite for lens fiber differentiation [63, 67].

Generally, cells undergoing mitochondrial outer membrane permeabilization (MOMP) do not survive, even if caspase activities are halted, due to a combination of mitochondrial failure and release of toxic proteins into the cytosol [68]. This intrinsic cellular process of mitochondrial leakage is termed caspase-independent cell death (CICD), which is mediated by MOMP [69]. More recent studies have implied that MOMP may be reversible in the presence of overexpressed glyceraldehyde-3-phosphate dehydrogenase due to its pivotal role in glycolysis and mitophagy [70]. The concept of anastasis opposes the basic framework of traditional chemotherapeutic agents and provides clues to better explain therapy-induced resistance in cancer. Understanding the detailed molecular processes during anastasis is vital to improve rational drug design and utilization.

4.2 Role of pro-survival and pro-metastatic genes: what is happening in anastasis?

As previously described, induction of cellular stress by chemotherapeutic agents causes activation of pro-survival genes which resist entry into apoptosis. This concerted effort to bar apoptotic signaling has traditionally been thought to only be effective before the apoptotic process is fully initiated [17, 65]. Recent reports suggest that the mediators of cellular survival can resurrect cells from late stages of apoptosis, thus defying the concept of a "point of no return" from apoptosis and providing an opportunity to study the complexities of anastasis [5].

Understanding of underlying molecular signature of anastasis originated from studies conducted in a mouse model wherein murine primary liver cells and NIH3T3 mouse embryonic fibroblast cells were exposed to ethanol as an apoptosis-inducing stressor. Following treatment cells were washed and cultured in fresh media to ensure recovery from ongoing apoptotic processes. Transcriptomic data analyzed during this recovery phase by using both RNA microarrays and qRT-PCR identify induction of many genes associated with survival pathways including members of *BCL-2* family (*BAG-3*, *BCL-2*, and *MCL-1*), *XIAP*, *MDM2*, and heat shock proteins (*Dnajb1*, *Hsp90aa1*, *Hspa1b*, and *Hspb1*) (Fig. 4) [8, 71]; moreover, the expression of residual apoptotic signatures disappears within 3–6 h of anastasis activation. However, inhibition of survival factors including Hsp90, MDM2, XIAP, and BCL-2 provokes abrogation of anastasis, thus implying the reliance on cellular survival mechanisms in anastasis. Survivability of cancer cells not only depends upon the expression of anti-apoptotic genes, although they play a significant part, but also on genes responsible for cell cycle regulation, DNA repair, angiogenesis, migration, and TGF-β modulation. These factors are found to be hyperactivated during early anastatic responses [8]. Furthermore, detailed study of molecular signatures of anastasis in HeLa cells following incubation with ethanol followed by recovery in fresh media incites a significant increase in the expression of *c-Fos*, *c-Jun*, *Klf4*, and *Snail-1* when analyzing the whole transcriptome by RNA sequencing (Fig. 4). The conclusion of this report implies that most of the hyperactivated genes during the initial recovery period after induction of apoptosis are related to the regulation of proliferation and pro-survival pathways [7]. In contrast, late anastasis gene signatures mostly included genes with an effect on actin cytoskeleton arrangements, ribosome biogenesis, protein processing, and pathways responsible for restoring focal adhesion (Fig. 4) [4, 7].

The elevation of survival factors, including MDM2, during anastatic response designates the possible involvement of p53 and its associated signaling counterparts such as cyclin-dependent kinases, E2F transcription factor, and cell cycle regulatory elements during the initial recovery period [72]. Further studies related to activation of AP-1 transcription factors including c-Jun and c-Fos are vital, as these genes can be either pro-apoptotic or pro-survival depending on the cellular context; therefore, anastatic activation of these transcriptional factors provides clues for the balance between pro-survival and pro-apoptotic mechanisms [73–75]. A majority of survival proteins involved in pro-proliferation and anti-apoptosis are also involved in recovery from the brink of cell death. This suggests the ability of cancer cells to activate survival mechanisms, even in extreme stress situations, resulting in major structural and molecular changes to revert to its normal physiological state.

Structural modifications during anastasis include activation of focal adhesion kinases and rearrangement in the actin cytoskeleton [5, 7]. These structural changes are highly correlated to cellular migration; thus, the cytoskeletal modifications during late phases of anastasis facilitate cellular migration in recovered cancer cells. Interestingly, Sun et al. have shown that *Snail-1* is highly induced during the early phase of anastasis; while its knockdown by shRNA clearly impairs the recovery process including migration, resulting in increased PARP-1 cleavage and persistence of cell death machinery. The authors also point out that the activation of TGF-β signaling during the early phase of recovery and its activation is well coordinated with cellular survival and migration. Apart from Snail, other EMT and angiogenic factors, *viz* matrix metalloproteases (MMP-9, MMP-10, MMP-13), VEGF-A, and Angpt-l4, have also been identified to be involved in recovery from cell death (Fig. 4) [7].

Cancer stem cells (CSC) are a matter of concern for the development of emerging therapies as they are highly linked with tumor relapse as well as drug resistance [76, 77]. Similarly, anastatic cells also represent a population of resilient cells resisting different phases of apoptosis. Recent

studies have shown that cells that recover from apoptosis display elevated expression of CD44, a prominent stemness marker, and possess cancer stem cell–like properties. Moreover, the reversal of apoptosis results in epigenetic changes in the promoter of CD44/CD24 [78]. Further studies to examine the implication of anastatic mechanisms in genesis of cancer stem cells are warranted.

In summary, anastasis utilizes the conventional pro-survival and pro-metastatic factors to hinder apoptotic progression. The activation of EMT and its associated modulators is not only associated with cellular survivability and metastasis but also extensively correlated with the stemness of cancer cells [79, 80].

5 Role of DNA repair mechanisms in EMT and anastasis

Faulty DNA repair mechanisms are deeply engrossed in carcinogenesis and contribute to the accumulation of mutations in the cancer genome [81]. DNA repair systems put a temporary halt on cell cycle progression which allows cells to repair damaged DNA sites. This also allows mechanisms to permit cells to escape from apoptosis and gain migratory properties since genes associated with cellular DNA repair system have been implicated in survival/EMT activation [82, 83]. For example, ATM kinase, a primary DNA damage sensor of double-stranded DNA breaks (DSB), facilitates NFκB release from IκB to execute its transcriptional role by phosphorylating NEMO (IKKy) at the ser^{85} site. This phosphorylated form of NEMO subsequently binds to ATM kinase and is exported to the cytosol where it interacts with other IKK subunits to trigger NFκB transcriptional activities [84–86]. Therefore, the activation of NFκB may elicit pro-survival responses mediated by upstream ATM kinase [87, 88].

ATM has also been implicated in mediating a pro-survival response by binding to AKT [89] as well as through positive regulation of a vital anti-apoptotic protein of BCL-2 family member, MCL-1 [90]. In addition to DNA repair, pro-survival, and anti-apoptotic mechanisms, ATM kinase also takes part in promoting EMT and cancer stemness. Recent research unveiled a novel role of ATM in the EMT cascade. In this study, inhibition of ATM kinase by KU-60019 resulted in disruption of vimentin-mediated activities, *viz* hindrance of ongoing apoptotic processes, and cellular migration *via* Snail-1 activation [17]. Snail-1 has also been identified to stimulate epithelial cells to enter EMT cascades once stabilized by ATM kinase [91]. Similarly, ATM stabilizes Zeb-1 which in turn activates radiotherapy resistance by stimulating DNA repair systems (Fig. 2) [92].

Poly (ADP-ribose) polymerase-1 (PARP-1) is a crucial DNA-binding protein involved in base excision repair (BER) and homologous recombination (HR). In addition to DNA repair functions, PARP-1 mediates several other tasks, including cellular proliferation and survival, which ultimately presents PARP-1 as a vital target for DNA damage response (DDR)–based therapeutics [93]. Briefly, PARP-1 can activate NFκB by modulating both CREB-binding protein and p300, and is also involved in the regulation of vimentin-mediated activation of Snail-1 and HIF1α gene transcription. Together, these actions further contribute to metastasis and angiogenesis [94].

DNA-damaging agents, including chemotherapy and radiotherapy, have demonstrated co-activation of DNA repair, cellular survival, and EMT; hence, its role in anastasis is also crucial. Recently, Tang et al. illustrated that ethanol-induced DNA damage is reversed during anastasis, resulting in the reversal of nuclear translocation of both AIF and Endo-G, both vital apoptotic nucleases which translocate to the nucleus during apoptosis. Consequently, PARP-1 cleaved during apoptotic signaling reverts back to its full-length form during anastasis while iCAD, inhibitor of caspase-activated DNase, levels are restored. This emphasizes the involvement of DDR proteins during anastasis [71]. DNA repair mechanisms have been linked with poor prognosis in cancer; the involvement of DNA repair proteins in anastatic processes warrants further study of the connection of these mechanisms in regard to therapy resistance [95].

6 Pro-survival responses associated with cancer metastasis and anastasis culminate in therapeutic resistance

Therapy-induced resistance is a major challenge to traditional chemotherapeutic approaches. Therapeutic failure accounts for significant morbidity and mortality in patients due to the development and selection of highly resistant, aggressive subclones mediated through the activation of pro-survival and metastatic signaling pathways [55, 96]. Proteins associated with pro-survival and DNA repair mechanisms, including members of the antiapoptotic proteins of BCL-2 family (BCL-XL, MCL-1, BCL-2), survivin, NFκB, c-FLIP, MDM2, and PARP-1, are extensively associated with cancer survival and corresponding drug resistance [97]. These molecular factors orchestrating resistance within the cancer cells also are also associated with the development of aggressive phenotypes including cancer "stemness," host immune response evasion, cellular dormancy, and migratory and metastatic potential [98, 99].

Anastasis is a process that encompasses both pro-survival and pro-metastatic activities; therefore, its role in drug resistance is inevitable. Although there are no direct reports that emphasize the involvement of anastasis in drug resistance, genes and processes associated with anastasis have been demonstrated to facilitate drug resistance (Table 1). For example, it was recently elicited that anastatic cells, having recovered

Table 1 Genes and processes associated with anastasis that have been demonstrated to facilitate drug resistance

S.no	Gene name	Definition	Function	Role in drug resistance	Reference
1	*ATF3*	Activating transcription factor 3	Cellular survival, proliferation, and differentiation	Radioresistance	[133, 134]
2	*c-FOS*	FBJ osteosarcoma oncogene	Cellular survival, proliferation, and differentiation	Early procurement of MDR phenotype.	[135]
3	*SOX-9*	SRY-box containing gene 9	Tumor growth, angiogenesis, differentiation, and survival	Chemoresistance against gemcitabine	[136, 137]
4	*c-JUN*	Jun proto-oncogene	Tumor growth, migration	Chemoresistance against sorafenib and increased expression in MDR cells	[137–140]
5	*INBHA*	Inhibin beta-A	Cancer cell proliferation	Chemoresistance	[141]
6	*SNAIL-1*	Snail family transcriptional repressor 1	Cancer cell metastasis and cellular survival	Drug and radioresistance	[142]
7	*ANGPTL4*	Angiopoietin-like 4	Oncogenesis, metastasis, and resistance to anoikis	Chemoresistance by raising the expression of ABC transporters	[143, 144]

from etoposide and paclitaxel treatment, demonstrated an increased expression of XPO-1, a nuclear export protein which is known to provoke acquired drug resistance in multiple myeloma [100, 101]. Cancer chemotherapy is currently based on the principle of periodic administration of drug [102]. Mounting evidences suggest that therapeutic approaches that allow cancer cells a recovery period may promote therapy resistance through mechanisms that have been associated with anastasis. Further study of anastasis as a drug resistance mechanism is warranted.

7 Combinatorial therapeutic approaches to attenuate pro-survival and EMT signaling

Anti-apoptotic mechanisms reviewed to this point, including EMT, DNA repair, and pro-survival pathways, cumulatively induce therapy resistance in cancer cells [103, 104]. The extensive use of DNA-damaging drugs clinically raises concern for the rapid development of resistance in tumors [57, 105]. Combinatorial approaches to prevent the pro-survival and pro-metastatic resistance processes are coming into vogue [106–108].

Pharmacological inhibition of the BCL-2 family of pro-survival modulators, *viz* flavopriridol, oblimersen, AT101, gossypol, and obatoclax, has demonstrated promising synergistic effects with different cytotoxic agents and has been studied in a wide range of tumors [109, 110]. Therapeutic approaches to mitigate pro-survival/anti-apoptotic factors, including survivin and XIAP, have also been shown to circumvent drug resistance [111, 112]. Pharmacological inactivation of Survivin by EZN-3042, an LNA antisense oligonucleotide (LNA-AsODN), enhances the effects of chemotherapeutic drugs in acute lymphoblastic leukemia (ALL) [113]. The small molecule targeting survivin gene transcription, YM155 (sepantronium bromide), in combination with carboplatin and paclitaxel is in phase I/II clinical trials [114]. Tetra-O-methyl nordihydroguaiaretic acid (M4N), another small molecule targeting Sp1-mediated transcription of survivin, amplifies the efficacy of temozolomide in glioblastoma cells [115], [116].

As described in previous sections, NFκB has been shown to confer a strong survival impetus. Therefore, NFκB-targeted therapy is rapidly emerging, bortezomib is reported to restrain NFκB by blocking the degradation of IκB, resulting in the abolition of NFκB signals. Application of this strategy in combination with other chemotherapeutic agents, *viz* temozolomide, paclitaxel, carboplatin, has exhibited promising results in preclinical studies [117]. Other inhibitors of NFκB, including curcumin, BMS-345541, DHMEQ, and bindarit (indazolic derivative), show potential for utilization in combination with DNA-damaging drugs to combat anastasis [118].

EMT is an important event in metastasis, anastasis, and chemoresistance, thus making it an attractive target for combinatorial drug design [59]. Molecules targeting EMT, including salinomycin, curcumin, mocetinostat, zidovudine, and metformin, have been extensively explored as agents to overcome chemotherapeutic resistance in combination with doxorubicin, 5-fluorouracil, docetaxel, and gemcitabine, respectively (Fig. 2) [57, 119–125]. HDAC inhibitors such as *n*-butyrate, trichostatin A (TSA), and vorinostat have effectively abrogated EMT through HDAC's role in suppressing the expression of E-cadherin as well as modulation of HIF-1α and NFκB expression [126–129]. In EGFR mutant cancer, diindolyl methane (DIM) and its analogs are reported to alter several pathways; however, its ability to block EMT and metastasis makes it an excellent candidate for combination with DNA-damaging agents [130, 131]. Recently, our group has demonstrated that DIM, in combination with camptothecin, abrogated drug-associated EMT in *Apc* floxed colorectal mouse models [17]. Similarly, combinatorial treatment with IKM5, an indolylkojyl methane analog, potentiates the efficacy of doxorubicin by eliminating the background GRP-78-mediated EMT activation [132]. Taken together, these results suggest that targeting EMT, through multiple mechanisms,

may represent a rational approach for combination with chemotherapeutic agents to address drug resistance.

8 Future directions

Research and development in cancer has evolved to such an extent that research has pivoted from identification of compounds with anti-cancer activity to deciphering the complexity of survival mechanisms and drug resistance at the molecular level. Studies are ongoing to further our understanding of molecular signaling between tumor and its microenvironment and how these crosstalk's influences, both cellular survivability and therapy-induced apoptotic resistance. The cellular mechanisms presented in this review collectively benefit cancer cells by reversal of apoptosis and induction of oncogenic phenotypes. The concept of anastasis represents a new paradigm in our understanding of cancer and challenges the basic understanding of cancer biology as well as the structure of traditional chemotherapeutic design. Advances in rational approaches to cancer therapy are necessary as the complexity of drug resistance is better understood. Thus, further research is warranted to develop personalized combinatorial treatment strategies that balance therapeutic efficacy with the burden of adverse events.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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