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Towards targeting of shared mechanisms of cancer metastasis and therapy resistance

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Abstract

Resistance to therapeutic treatment and metastatic progression jointly determine a fatal outcome of cancer. Cancer metastasis and therapeutic resistance are traditionally studied as separate fields using non-overlapping strategies. However, emerging evidence, including from in vivo imaging and in vitro organotypic culture, now suggests that both programmes cooperate and reinforce each other in the invasion niche and persist upon metastatic evasion. As a consequence, cancer cell subpopulations exhibiting metastatic invasion undergo multistep reprogramming that — beyond migration signalling — supports repair programmes, anti-apoptosis processes, metabolic adaptation, stemness and survival. Shared metastasis and therapy resistance signalling are mediated by multiple mechanisms, such as engagement of integrins and other context receptors, cell–cell communication, stress responses and metabolic reprogramming, which cooperate with effects elicited by autocrine and paracrine chemokine and growth factor cues present in the activated tumour microenvironment. These signals empower metastatic cells to cope with

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Author contributions

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therapeutic assault and survive. Identifying nodes shared in metastasis and therapy resistance signalling networks should offer new opportunities to improve anticancer therapy beyond current strategies, to eliminate both nodular lesions and cells in metastatic transit.

Cancer metastasis depends upon a sequence of interdependent steps by which cancer cells migrate and evade from the primary tumour, engage with peritumoural stroma, penetrate blood vessel walls, withstand shear stress in circulation and adapt to the micromilieu of a distant organ[1,2]. Each step involves mechanochemical sensing from a range of environments, and cell activation by chemokines and growth factors[3,4]. Cancer cells respond to these signals to execute cytoskeletal dynamics, adhere and migrate. The cells further receive signalling from the extracellular matrix (ECM), which increases their ability to repair cell organelles and DNA and thereby reduce apoptosis signalling and survive[5]. Invasion-associated signals further cooperate with longer-lasting response programmes in cancer cells, such as the `epithelial-to-mesenchymal transition` (EMT) and metabolic stress responses, which jointly provide an array of signalling networks that control cell growth, differentiation and stemness[2,3,5].

Originally recognized in 2012 (REF.[6]), the crosstalk between invasion and metastasis programmes and therapy resistance has been consolidated using multitargeted therapy that is effective in targeting both programmes in 3D organotypic culture and preclinical animal models. In addition, circumstantial evidence from clinical studies supports the concept of shared vulnerabilities that can be exploited to improve outcomes in patients. `Adaptive resistance` mechanisms that are triggered by cancer cell invasion and metastasis comprise (1) cell-intrinsic mechanochemical networks mediating migration, (2) matrix-derived constitutive signals encountered by metastatic cells when they are moving through tissue compartments and (3) paracrine survival signals delivered to metastatic cancer cells from responsive cells residing in secondary microenvironments. Potentially, this crosstalk can contribute to the evolution of particularly detrimental cell subpopulations possessing enhanced metastatic and survival abilities. Cells undergoing metastasis can consequently be considered as a mobile niche throughout the body, which produce clones with particularly strong survival abilities[2,7,8].

In this Perspective, we briefly review the cell-autonomous and microenvironmental signalling pathways active in cancer cells from solid tumours during invasion and metastasis, and summarize mechanisms of therapy resistance. We then discuss how invasion programmes undergo crosstalk with cancer cell survival and therapy resistance signalling. A central theme within this is the production of stroma-derived signals, which induce pre-emptive cell fitness with accelerated DNA damage response programmes, changes in cell cycle control and anti-apoptosis signalling; these programmes enable metastatic cancer cells to cope with environmental as well as drug-induced cell stress. Ultimately understanding the crosstalk between metastasis and therapy resistance programmes will allow identification of intervention points and therapy strategies to combat metastatic progression and overcome therapy resistance in an integrated approach.

Signalling in invasion

During metastatic dissemination, cancer cells integrate cell-intrinsic and microenvironmental stimuli together with adaptation responses to accommodate changing tissue conditions and local stresses. Cell-intrinsic signalling and cytoskeletal programmes are engaged to execute migration as individual cells or multicellular groups of connected cells[2]. The maintenance of cell plasticity during cancer progression strongly determines the metastatic capability[9,10]. Specifically collective dissemination has recently been recognized as an effective mechanism of metastasis, on the basis of strongly enhanced prosurvival signalling and, potentially, therapy resistance identified in 3D invasion models and in vivo[11,12] (BOX 1). Invading cancer cells interact with tissue structures and receive cytokine signalling from microenvironments, which differ between tissues and tumour sites[13]. Migration through tissue and across tissue boundaries further imposes mechanical and chemical challenges upon tumour cells, which is followed by an adaptive stress response to counter the damage[14,15]. Through these combined signals, tumour cells navigate through tissue, change their cell shape and stability of the cell body and nucleus, and adjust their intracellular metabolism to extracellular conditions[16–18].

Cell-intrinsic programmes

Migrating cells exhibit active signalling networks that control cytoskeletal activity and achieve front–rear polarization, changes in shape and turnover of cell adhesion sites in response to the extracellular environment. In solid tumours, the small GTPases RAC1, CDC42 and RHOA, in concert with receptor transmembrane signalling, control actin polymerization, and the contraction of actin filament networks by myosin motors. Consequently, upstream or downstream effectors of Rho GTPase pathways, such as Rho-associated kinase (ROCK), represent targets for pharmacological intervention. In addition, cell–cell interactions between tumour cells support homophilic cell–cell signalling and multicellular cytoskeletal coupling and enhance metastasis[12,19].

Homotypic interactions between tumour cells.

During collective invasion and metastasis, tumour cells retain cell–cell adhesion and mechanical connections by adhesion molecules and gap junctional intercellular communication[19,20]. Cell interactions determine multicellular guidance[20,21], stabilize circulating tumour cell (CTC) clusters in the bloodstream[22] and enhance metastatic organ colonization[12].

E-cadherin mediates cell–cell adhesion and collective metastasis in highly differentiated epithelial cancers, whereas E-cadherin, N-cadherin and other cadherins maintain adhesions in mesenchymal tumours and cells that have undergone partial EMT[12,23] (FIG. 1a). Adherens junctions inhibit RHOA signalling and actomyosin contractility along the cell–cell contact, which reduces tension and keeps the moving cell group intact[24]. Other cadherins, including N-cadherin and P-cadherin, result in cell–cell interactions of lower stability but still support collective metastasis[25]. Importantly, junctional cadherins inhibit progression of the cell cycle by antagonizing β-catenin–WNT signalling and the `Hippo pathway`, and

by retaining Yes-associated protein (YAP) in the cytosol[26] (FIG. 1a). Besides cadherins, additional cell–cell adhesion systems are being implicated in collective metastasis (BOX 2).

Autocrine stimulation.

Tumour cell-derived release of promigratory factors, including chemokines, growth factors and nucleotides, activates autocrine receptor-mediated downstream signalling to induce cell polarity and single-cell or collective invasion[20,27]. The tight proximity of neighbouring tumour cells enables effective concentrations of autocrine and paracrine growth factors to accumulate via `nanolumenal release` and induce growth factor receptor signalling (for example, epidermal growth factor receptor (EGFR))[28]. Autocrine signalling cooperates with paracrine release of cytokines from the tumour stroma (discussed later).

Microenvironmental programmes Chemotactic and cytokine signalling.

Soluble factors released by tumour cells or the microenvironment activate promigratory signalling, and thereby induce and maintain cancer cell invasion[3,13]. Chemokines, including CXC-chemokine ligand 12 (CXCL12; also known as SDF1) and CC-chemokine ligand 2 (CCL2), along with bioactive `lipid mediators` and adenosine nucleotides induce directional migration towards the source of the factor[29]. Chemokine signalling through G protein-coupled receptors (GPCRs) induces PI3K–AKT activation, which in turn engages RAC1 and actin polymerization, leading to the formation of a leading edge[20,30,31] (FIG. 1b). Growth factors, including fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF) and epidermal growth factor (EGF), released by stromal cells induce cancer cell migration[31,32]. Canonical receptor tyrosine kinase (RTK) signalling, through MAPK, PI3K–AKT and Rho GTPases, activates cytoskeletal activity as well as other signalling pathways, such as the mTOR complex (mTORC), which regulates motility, growth and other cellular functions[32] (FIG. 1b). Further promigratory signalling is delivered by receptors for stromal signals, including transforming growth factor-β (TGFβ), bone morphogenetic proteins (BMPs) and heparin-binding EGF (HBEGF), as well as growth arrest-specific protein 6 (GAS6) via `cell detritus` originating from cell death processes in the tumour microenvironment (TME)[33] (FIG. 1b). Despite not being chemotactic themselves, cytokines can facilitate non-directional migration, which leads tumour cells towards guiding tissue structures, such as myofibres, vessels and nerves[34].

Context receptor signalling.

`Integrin` adhesion receptors bind to ECM including collagen, fibronectin, laminin and other ligands, and connect mechanically to the actin cytoskeleton through cytoskeletal linkers[35]. In turn they activate small Rho GTPases and the actin cytoskeleton by recruiting a broad range of intracellular adaptor proteins and kinases[36] (FIG. 2). As an example, focal adhesion kinase (FAK) and SRC activate PI3K–AKT and RAS–ERK signalling, which, through RHOA, RAC1 and CDC42, control actin dynamics and migration[37,38]. Integrins further activate the transcription factor nuclear factor-κB (NF-κB) and activate matrix metalloproteinases (MMPs), which support local tissue invasion and ECM degradation[37]. The quality and strength of integrin-mediated adhesion signalling depends on the local ECM

density and stiffness and cell contractility[39]. Non-integrin adhesion receptors also contribute to cancer cell adhesion and signalling during metastasis (BOX 2). Context receptors, in concert with stromal signals, such as TGFβ and WNT, engage the Hippo pathway[40,41]. This pathway leads to nuclear translocation of YAP and transcriptional co-activator with PDZ-binding motif (TAZ; also known as WWTR1) and concomitant co-activation of the transcription factors T cell factor/lymphoid enhancer-binding factor (TCF/LEF) and SMAD[42] (FIG. 2). In metastatic cancer cells, Hippo signalling induces RHOA signalling and actomyosin contractility, prevents apoptosis and induces metabolic adaptations required for dissemination[43].

Survival and resistance signalling

An ability to survive environmental and drug-induced damage is central to cancer progression and therapy resistance. As part of neoplastic transformation, survival strategies may become clonally selected, resulting in primary drug resistance. In addition, cancer cell survival signalling is adaptive. Signalling networks cooperate with oncogenic signals, respond to microenvironmental cues and drug-induced perturbations, and consequently may increase cell fitness, cell survival and drug resistance.

Membrane repair

To secure survival, nucleated cells repair damaged plasma membrane or nuclear membrane. Mechanical stress, including cell compression by rigid ECM structures or fluid shear stress, can cause rupture of the nuclear and plasma membranes. Membrane defects are repaired, within minutes to hours by the `endosomal sorting complexes required for transport III` (ESCRTIII) machinery and annexin A7 (REFs [14,44]). ESCRTIII and annexin A7 form a complex that marks the defect, recruits intracellular vesicles as a membrane source and closes the defect by budding and shedding of the damaged part[45] (FIG. 3a). Membrane repair minimizes uncontrolled leakage of cytoplasmic or nuclear content (for example, of cytoplasmic endonucleases, which can degrade DNA and induce a DNA damage response[46]). To prevent membrane rupture in mechanically challenging environments, actomyosin networks increase local stiffness and stability of the cell[14] (FIG. 3a). Within minutes, cytoskeletal stiffening and membrane repair are frequent activities that prevent long-lasting structural damage, particularly in invading tumour cells and CTCs[47].

DNA repair

The reorganization of nuclear DNA and genomic instability are both causes and consequences of cancer, and have an important impact on tumour cell survival and therapy response[48] (FIG. 3b). DNA double-strand breaks and damage are induced by mechanical stress, reactive oxygen species (ROS) and exposure to radiation and chemotherapy. DNA damage sterically impairs DNA transcription and RNA polymerases and thus gene expression or DNA replication[48]. Depending on the type of damage, DNA repair is a multistep process that leads to the formation of a repair complex consisting of ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related protein (ATR) and other proteins; for example, ligases that reconnect the disrupted DNA strand[49]. ATM and ATR also engage p53 signalling to upregulate expression of survival-enhancing genes

such as *MDM2*, the protein product of which inhibits the transcriptional activity of p53 by degradation[50] and cyclin-dependent kinase (CDK) inhibitor p21, which regulates cell cycle checkpoints through binding to CDKs[51]. To repair DNA without perturbing gene transcription, cell cycle checkpoints inhibit proliferation until the DNA is repaired, typically within hours to days, and cell growth then resumes[52]. If persistent signalling via ATM and ATR, p53 and CDK inhibitors (for example, p16 and p21) perpetuates the cell cycle arrest, dormancy is induced. Alternatively, when DNA damage is combined with telomere shortening[53], a combination of dormancy and senescence is induced[54].

Avoidance of apoptosis induction

If DNA repair is unsuccessful, DNA damage mediators (for example, BRCA1, the histone variant γH2AX and p53-binding protein 1 (53BP1))[55,56] induce apoptosis signalling via the ATM and ATR pathways and checkpoint kinase 1 (CHK1)-mediated and CHK2-mediated p53 signalling[49] (FIG. 3c). These pathways induce transcription of proapoptotic genes[57], and activate multiple pathways to apoptosis. Mitochondrion-dependent apoptosis is induced by p53-mediated upregulation of BCL-2 family proteins[58], which induce caspase-dependent permeabilization of the mitochondrial membrane and cell death[59]. Apoptosis can also be induced by p53-dependent induction of death receptors and ligand expression at the cell surface, including FAS and FAS ligand (FASL)[60]. FAS–FASL-mediated apoptosis depends on contacts made between neighbouring cells followed by activation of caspase 8 and caspase 10 ([REFs 61,62]) (FIG. 3c). In contrast to normal cells, cancer cells can escape apoptosis despite persistent DNA damage and new genomic alterations. For example, overexpressed `survivin` (also known as BIRC5) constitutively inhibits caspases and ensures cell survival, even when DNA damage is not repaired[63]. Likewise, oncogenic signalling can disrupt controlled apoptosis. For example, deletion or functional inactivation of p53 or effectors of DNA repair or cell cycle control, including RB, p21 and p27, impairs apoptosis induction[64].

Decreasing drug efficacy

Tumours, even when initially susceptible to anticancer therapy, can reduce cytotoxic effects by lowering the intracellular activity of the drug, through chemical inactivation or by secreting the drug into the extracellular space[65]. ATP-binding cassette (ABC) efflux transporters in the plasma membrane, including P-glycoprotein (P-gp), multidrug resistance-associated protein 1 (MRP1) and breast cancer resistance protein (BCRP), clear anticancer drugs from the cytoplasm, reduce the locally active concentration and thereby limit the effectiveness of chemotherapy[66]. Consequently, upregulation of drug efflux transporters in response to drug treatment can contribute to therapy resistance[65].

Escape from immunosurveillance

Tumour cells develop a range of immune escape strategies, which reduce immune cell effector function[67]. Either constitutively or in response to mechanochemical stress and hypoxia, tumour cells downregulate major histocompatibility complex class I (MHCI) at the cell surface and thereby reduce both antigen-dependent activation of T cells and elimination by cytotoxic CD8+ T lymphocytes (CTLs)[68]. MHCI downregulation occurs

through epigenetic silencing and transcriptional repression controlled by growth factor and WNT–β-catenin signalling[69]. Similarly, epigenetic silencing of the proteasome complex reduces antigenic peptide delivery to MHC as well as MHC–antigenic peptide expression at the cell surface[69]. MMPs and other proteases in the TME cleave and shed MHCI, adhesion molecules (intercellular adhesion molecule 1 (ICAM1)) and death receptors (FAS and tumour necrosis factor-related apoptosis-inducing ligand (TRAIL)) on tumour cells, which reduces recognition by CTLs[68,70]. These immune escape programmes predispose tumour cells to avoid immune-mediated recognition during neoplastic evolution. In addition, a broad range of cytokine signals and regulatory immune checkpoints contribute to reprogramming of the TME and immune escape[67,71]. For example, tumour cells that have undergone EMT together with tumour-associated macrophages and fibroblasts release immunosuppressing cytokines, including TGFβ and interleukin-10 (IL-10)[72], which inhibit effector functions of CTLs, natural killer (NK) cells and effector macrophages[71]. Paracrine cytokine signals further upregulate immunomodulatory surface ligands on tumour cells, including cytotoxic B7-1, B7-2 and programmed cell death ligand 1 (PDL1), which activate inhibitory receptors on T cells and thereby dampen the activity of CTLs and NK cells[67]. Immunosuppressive metabolites of the reactive tumour stroma, including adenosine and lysyl oxidase (LOX) impair CTL effector function and upregulate immunosuppressive, regulatory T cells[73].

Environmental control of survival

The survival programmes discussed so far are regulated by adhesion and cytokine signals and metabolic challenges experienced by tumour cells during invasion and metastasis as well as therapy-induced stress. Adhesion receptor signaling through integrins, CD44 and syndecans as well as ECM-binding RTKs mediates prosurvival signalling, and perturbation of their activity causes `anoikis`[35]. Stromal cytokines can activate transcriptional survival programmes. Signalling pathways activated by GPCRs and RTKs, including the PI3K–AKT–mTORC, Janus kinase (JAK)–signal transducer and activator of transcription (STAT), RAS–MAPK, protein kinase A (PKA) and PKC pathways, activate the transcription factors SLUG, SNAI1, TWIST, ZEB1/ZEB2, FOS–JUN, NF-κB, JAK–STAT, forkhead box O3 (FOXO3) and SMADs, which form a coordinated signalling network coordinating integrated prosurvival responses, including EMT, glycolysis, autophagy and anti-apoptotic programmes[4,13,74]. Cancer cell survival can also be promoted by oestrogen and androgen signalling[75]. Hypoxia and acidosis cause activation of pathways that regulate cell metabolism, including hypoxia-inducible factors (HIFs), AMP-activated protein kinase (AMPK) and mTORC, and furthermore inhibit BCL-2-mediated cell death pathways[76,77]. Mechanical stress experienced during invasion activates the Hippo pathway and transcriptional cell reprogramming through adherens junction and integrin signalling[78]. In response to DNA damage, activation of the poly(ADP-ribose) polymerase 1 (PARP1) and AMPK pathways[79] and p53-induced transcription of PTEN, an antagonist of PI3K–AKT–mTORC signalling, lead to activation of autophagy, which enables alternative sources of energy and secures survival[48,80,81].

Besides adaptive and relatively transient (hours to days) signalling and transcription regulation, oncogenic and microenvironmental signals can cause sustained reprogramming of cancer cells for weeks and beyond, through epigenetic regulation. DNA-modifying

enzymes and histone-modifying enzymes regulate the accessibility of chromatin for transcription factors, the DNA repair machinery and histone-modifying enzymes themselves, and cause long-lasting adaptations of gene expression (FIG. 3d). In cancer cells, DNA methyltransferases (DNMTs) can be activated by, for example, MYC, RAS–activator protein 1 (AP-1), MEK, JUN amino-terminal kinase (JNK)–SP1 or JNK–SP3 ([REFs 82,83]). As an example, DNA methylation can repress expression of E-cadherin and FOXO proteins, inducing EMT and stemness, respectively[84,85]. Histone deacetylases (HDACs) can support or inhibit apoptosis, by downregulating BCL-2 or activating p53 and BAX, respectively[86]. In cancer, activated histone acetyltransferases (HATs) upregulate oncogenic and survival-enhancing gene expression, including expression of the genes encoding MYC, STAT5 and PARP1 (REF.[87]). In response to oncogenic signalling, through CDK4, SNAI1/2 and YY1 (REF.[88]), chromatin hypermethylation performed by histone methyltransferases (HMTs) represses a range of survival-suppressing genes, including *CDKN1A*, *CDKN2A*, *KRAS* and *MAPK1*[89,90].

Cooperation of signalling programmes

Given that both cell migration and cell survival pathways are engaged by the myriad of signalling pathways activated in diverse TMEs, increasing interest is being focused on the mechanisms linking invasion and metastasis processes with altered response to anticancer therapy. Along with individual pathway influences on both migration and survival, significant crosstalk exists among the various pathways, which often limits the efficacy of targeting individual pathways and mandates combination therapy.

Stress and repair responses engaged during tissue invasion and in CTCs activate cytoskeletal responses and favour dissemination and metastasis. The adhesion pathways mediating cell survival strongly overlap, or are identical to, their invasion-promoting signalling, including FAK–SRC signalling, RHOA–ROCK and PI3K–AKT, and transcriptional reprogramming through NF-κB, YAP/TAZ and mTORC pathways[35,36,43]. Mechanical, thermal, chemical and metabolic challenges, as well as DNA repair signalling, can induce an intracellular stress response, which simultaneously activates the cytoskeleton, intracellular signalling, transcriptional regulation and metabolic adaptation. Likewise, nutrient deprivation and therapy stress can induce an EMT-like state, which includes a metabolic switch to glycolysis and autophagy, cell cycle arrest and cytoskeletal activation. While each pathway supports cancer cell survival independently, these context-dependent pathways may support survival in invading and metastatic tumour cells as an integrated programme[74]. This joint programme ensures cell survival and induces migration and escape from the stressful environment[91]. As a consequence, this reciprocity between invasion and survival signalling enables metastatic cancer cells to use repair mechanisms and cope with stresses from both (1) mechanochemical effects and (2) cytostatic, cytotoxic or molecularly targeted treatments, and yield enhanced resistance to therapy.

An important link generating long-lived induction of invasion and metastasis-associated therapy resistance programmes is HDACs. HDACs are upregulated in cancer cells by MAPK and JNK signalling, via the transcription factor SP1 (REF.[92]) and control chromatin remodelling involved in invasion programmes as well as DNA repair and survival (FIG.

3d). For example, HDACs enhance the autocrine release of IL-8 (REF.[93]), G1 cell cycle arrest[94] and invasiveness via AKT and MAPK signalling[95,96]. Histone-modifying enzymes also modify non-histone proteins, and thereby regulate the function of transcription factors, scaffold proteins and cytoskeletal proteins directly[97]. As an example, HDAC6 acetylates tubulin in epithelial and mesenchymal cells, and this increases cell–ECM adhesion, cell spreading, and turnover of adhesions and invasion[98].

Understanding of these coupled consequences is being catalysed by applications of advanced 3D tissue culture models and live-cell and intravital microscopy. The crucial feature of these technologies beyond more traditional methodological approaches is their intrinsic incorporation of complex microenvironmental influences on the tumour cells, and vice versa. In practice, these advances are enabling detection of the intersection of invasion and survival processes in response to therapeutic challenge and/or molecular interference in context, including the underlying mechanisms of a cancer cell's enhanced abilities to repair DNA damage, accommodate metabolic demands and develop features of anchorage independence and stemness (see Supplementary Table 1).

Enhancing therapy resistance

Key signalling pathways involved in cancer cell invasion also mediate drug resistance. These include GPCR and RTK signalling, context receptors mediating cell–cell and cell–ECM interactions, and environmental stress (see Supplementary Table 1). Activation of the chemokine receptors CXC-chemokine receptor 4 (CXCR4) and CXCR7 by CXCL12, or chemotactic lipid mediators, including leukotrienes and prostaglandins, through PI3K–AKT, phospholipase C (PLC) and MAPK induces resistance of cancer cells to chemotherapy and radiotherapy in vitro and in vivo[31,99]. Similarly, migration-inducing RTKs, including EGFR, FGF receptor (FGFR), insulin-like growth factor 1 receptor (IGF1R) and AXL, primarily through MAPK/ERK signalling but also through PI3K–AKT, NF-κB and JAK–STAT signalling, induce resistance of cancer cells to chemotherapy, radiotherapy and molecular therapies[100,101]. Apoptotic cell debris containing phosphatidylserine as well as GAS6 are products of therapy-induced tumour cell death. Confoundingly, these products activate the AXL signalling pathway, which increases tumour cell migration[33]. As a consequence, therapy-induced cell death and AXL pathway activation have been implicated in supporting tumour repopulation and metastatic evasion of tumour cell subsets that survived therapy[33,102]. Besides cell death, stromal activation in response to oncogenic (that is, oncogenic KRAS-mediated) cancer cell signals reciprocally activates the AXL and IGF1R pathways and other pathways[103]. The migration-inducing factor HGF, released by activated macrophages, activates MET–CD44 and downstream JNK–JUN signalling, which supports tumour cell survival and reduces sensitivity to MAPK inhibitors[101]. As a consequence of invasion induction, activated RHOA–ROCK–myosin II signalling, which is required for cytoskeletal activity, also dampens ROS induction, supports DNA damage repair and upregulates immunosuppressive PDL1 in tumour cells, and thereby increases resistance to MAPK inhibition and immunotherapy[104].

Activation of integrins, through engagement of FAK, SRC, AP-1, PI3K–AKT and MAPK signalling, prevents anoikis in migrating cells[105,106] and induces resistance to

radiotherapy[107,108]. As an example, during cancer cell invasion, integrin engagement facilitates accelerated DNA repair upon radiotherapy[11]. Inhibition of integrin-mediated reprogramming induces radiosensitization and elimination of metastasis[11]. Integrins mediate resistance by upregulating survival-promoting cyclin D1 kinase[109] and repressing proapoptotic FOXO[35]. Similarly and potentially overlapping with this mechanism, the actin-bundling protein fascin, which is engaged in filopodia of invading cancer cells, also activates PI3K–AKT, followed by anti-apoptotic signalling and chemoresistance[110]. FAK can also shuttle into the nucleus and induce degradation of p53, stabilize nucleostemin, a cancer stem cell marker, and contribute to the stress response[111]. Furthermore, increased actin polymerization (for example, upon integrin activation) releases myocardin-related transcription factors (MRTFs) from G-actin[112]; these in turn modulate the cell cycle and stemness, through cyclin D1, MYC and NANOG, OCT4, paired box 6 (PAX6), SRY-box 2 (SOX2) and nestin[113]. Integrin–ECM interaction activates ABC efflux transporters, which decreases intracellular drug concentration and mediates chemoresistance to doxorubicin and mitoxantrone[114]. In line with this, CD44, alone or in cooperation with MET receptor signalling, upregulates P-gp (a multidrug resistance protein) and BCL-2 expression and thereby promotes chemoresistance[115]. In addition, CD44 upregulation or interaction with hyaluronan activates MAPK signalling and induces autophagy, followed by chemoresistance[116]. Chemoresistance can also be induced by discoidin domain-containing receptor (DDR) engagement with collagen through MAPK signalling, NF-κB and cyclooxygenase 2 (COX2) activity[117]. Thus, context receptors jointly mediate tumour cell invasion and enhance resistance to anticancer therapy.

Mechanochemical stress signals experienced by metastatic cancer cells can, perhaps surprisingly, enhance therapy resistance. One example of this is Hippo signalling through YAP activation, which along with supporting EMT helps mediate independence from oncogenic MAPK signalling and resistance to MAPK inhibitor treatment[118–120]. Similarly, HDAC regulation and `chromatin organization` are sensitive to mechanical stress. For instance, stiff ECM induces the expression of HDAC3 and HDAC8, accompanied by chromatin remodelling[121], and HDAC3 nuclear localization is regulated by RHOA–ROCK signalling[122]. As a consequence of HDAC and HAT regulation, chromatin remodelling supports acquired radioresistance[123].

Metabolic stress, besides inducing a metastatic escape response[18,91], also activates stress response signalling and augments therapy resistance of cancer cells. As an example, hypoxia signalling can cause resistance to PI3K pathway inhibition by rapamycin; however, this adverse event can be therapeutically reversed by combining mTORC1 and mTORC2 inhibition with hypoxia-activated chemotherapy (TH-302)[124]. In response to metabolic stress, autophagy induction in migrating cancer cells results in the release of ATP into the extracellular space. Extracellular ATP, in turn, activates purinergic receptors and downstream PI3K and MAPK signalling, and upregulates drug efflux and stemness genes[125]. Consequently, purinergic receptor inhibition, which dampens the paracrine ATP signaling in response to autophagy stimulation, reduces the development of resistance to BRAF inhibition in metastatic melanoma cells[125]. The mechanisms by which migration-enhancing microenvironmental signalling, the duration of which is typically transient (in the

range of hours to days), leads to more sustained therapy resistance in cancer cells remain to be further explored. Therapy-induced DNA damage has been found to yield survival of minor subpopulations of invading tumour cells, allowing them to accumulate new mutations resulting in clonal escape[8]. For instance, cancer cells escaping combination radiotherapy and targeting of integrins and EGFR show new mutations in genes mediating survival and therapeutic resistance signalling, which in turn leads to the activation of mTOR signalling and autophagy induction through an antioxidative stress response mediated by Kelch-like ECH-associated protein 1 (KEAP1)–NFE2-related factor 2 (NRF2) signalling[126,127].

In addition to the introduction of mutations, therapy can activate stress-related pathways, which regulate chromatin and histone-modifying enzymes, to cause epigenetic reprogramming that is retained for days and weeks[128]. Examples include YAP–TAZ signalling[43], autophagy[129] and EMT signalling[90], which all introduce multiparameter programmes of drug resistance by several, often combined outcomes: (1) delay or cessation of cell cycle progression[130], (2) induction of stemness features and (3) metabolic adjustment to glycolysis and autophagy[90]. Microenvironmental and epigenetic reprogramming can ultimately induce longer-lasting EMT-like programmes as well as autophagy, which both downregulate cell–cell adhesion, increase focal adhesion formation and turnover, and increase local invasion and metastasis[131,132].

Resistance coupled to metastasis

Stress response signals that mediate repair and cell survival may induce migration, the duration of which likely depends on the type and duration of the upstream signal and the extent of epigenetic reprogramming, lasting from hours to days, or beyond. Surviving cancer cells resisting therapy retain their ability to migrate and, hence, can escape from the damaged site. In response to therapy stress, RTK signalling, including through MET and AXL, can be upregulated[133] and increase cell responsivity to external signals, including to the motility-enhancing ligands HGF and GAS6 (REF.[134]). Increased DNA methylation in response to therapy stress, through activation of DNMTs, can induce both chemoresistance and invasion programmes by repressing tumour suppressor genes[135], including those encoding E-cadherin and `tissue inhibitor of metalloproteinases 3` (TIMP3)[84,136].

Cytotoxic therapies, besides inducing stress and death in tumour cells, can also activate the TME. Chemotherapy and radiotherapy upregulate migration-inducing factors in the TME, including CXCL12, CXCR3 ligands and CCL5, as well as lipid mediators and growth factors, including EGF, HGF and FGF, and RTKs[30,137–139], which can trigger cancer cell invasion[140], intravasation into the blood vasculature and circulation, and distant metastasis[141]. Macrophages, activated by chemotherapy stress, express CXCR4, release EGF and promote invasion and metastatic evasion of breast cancer cells[139,141,142]. Likewise, fibroblasts activated by radiotherapy induce a stromal wounding response, followed by deposition of fibrillar type-I collagen, which in turn enhances the invasiveness of colon cancer cells[143]. Additional invasion-enhancing proteins released by fibroblasts in response to chemotherapy include thrombospondin 1 and secreted protein acidic and rich in cysteine (SPARC)[144]. Furthermore, cancer-associated macrophages and fibroblasts, when activated

by chemotherapy, release factors that can enhance survival of cancer cells, including IL-8, CXCL2 and MMPs[145], which are known to contribute to EMT development and activation of DNA repair pathways[3].

Crosstalk of signalling pathways

Owing to the complex interactions within signalling networks, metastasis-associated resistance can be initiated by different, often concurrent events, cues and perturbations. Whereas experimental analysis typically focuses on individual pathways, their cooperation resulting in additive, synergistic or antagonistic effects is poorly understood. Signalling pathways that steer cell migration intersect with signalling pathways controlling other cell functions, including cell cycle progression and cell differentiation (FIG. 4). In cancer cells, this crosstalk can modulate the invasion mode and metastatic outcome, as well as growth and survival programmes.

Chemokine receptor and RTK downstream signalling elicits a broad range of short-lived and potentially long-lived downstream effects. PI3K–AKT signalling inhibits transcription of proapoptotic genes, including the genes encoding FOXO, caspases and BCL-2-associated agonist of cell death (BAD); in parallel PI3K–AKT signalling enhances anti-apoptotic signalling, either by transcriptional regulation or by post-translational regulation of downstream effector genes[146]. Activation of NF-κB through AKT upregulates the inhibitors of apoptosis survivin and XIAP[147], and further supports autophagy, by upregulating beclin 1 ([REFs 148,149]). MAPK pathways engaged in migrating cells also activate transcription factors, including cAMP-responsive element-binding protein (CREB), MYC and NF-κB, promoting survival and proliferation in cancer cells[150]. PI3K–AKT signalling also activates RAC1 and RELA, and upregulates cyclin D1 expression, which promotes cell cycle progression[151]. Thereby, chemokines and cytokines mediate integrated signaling networks that steer invasion, growth and survival in combination.

Integrins transactivate RTK signalling, through FAK, PINCH1 and integrin-linked kinase (ILK), even in the absence of growth factor ligand[152]. When stimulated by extracellular ligand, RTKs, including platelet-derived growth factor receptor (PDGFR), FGFR, vascular endothelial growth factor receptor (VEGFR), EGFR, MET and AXL, enhance integrin-mediated adhesion and signalling, which results in reciprocal overactivation of both pathways. As an example, stimulation of MET by HGF or members of the EGFR family (for example, EGFR and ERBB2; also known as HER2 and NEU) together with integrin signalling enhances cancer cell transformation and growth and induces tissue invasion[153–155].

Integrins and other migration-regulating pathways, such as E-cadherin, can also impact transcription factor translocation to the nucleus directly, by modulating the scaffolding function of the actin cytoskeleton and thereby crosstalk with survival and stemness pathways[156]. Signalling crosstalk engaged during invasion can also occur between adhesion systems. E-cadherin downregulation can be induced by growth factor-mediated transcriptional regulation or cleavage of E-cadherin by extracellular proteases, resulting in partial EMT[157]. Moderate lowering of E-cadherin expression activates the SRC, ILK and RAC1–RHOA pathways, which, in turn, accelerates focal adhesion turnover and invasion[158].

Similarly, engagement of integrins reduces cadherin-mediated cell–cell adhesion. As an example, exposing immortalized but not neoplastic mammary epithelial cells to stiffened ECM induces integrin engagement, rearrangement of E-cadherin and reduction of apicobasal polarity, followed by invasion[159]. Here, the cadherin and integrin pathways cooperate and mediate collective invasion, with intact cell–cell contacts[159], a phenotype typically observed in invasive cancers in the clinic[160] (BOX 1). Hence, a major challenge for experimental research lies in dissecting the hierarchy of such signalling networks to identify central signalling ‘hubs’ that can be targeted therapeutically to inhibit metastasis and survival programmes to an equal extent.

Reactivating morphogenesis pathways

Many of the pathways activated during cancer invasion and metastasis recapitulate morphogenesis programmes, which control cell growth, migration and positioning during embryonic development. Ligands, receptors and signalling intermediates of the EGF, IGF, FGF, HGF, WNT, TGFβ and PDGF families are expressed throughout embryonic development, where they control both cell migration and cell survival pathways[19,161]. As an example, in early embryogenesis IGF1R, in cooperation with E-cadherin, induces glycolytic pathways and anti-apoptotic responses through PI3K–AKT–RHOA signalling[162,163]. The PI3K–AKT pathway further regulates the EMT processes underlying neural crest cell migration, epithelial sprouting and mesoderm formation[164]. Integrin and cadherin interactions and signalling mediate epithelial and mesenchymal cell–matrix interactions and cell–cell cohesion required for collective movements that underlie gland, duct and organ development, including liver, kidney, lung and muscle formation[4,165]. Arguably, the reactivation of morphogenesis pathways in neoplasia equips cancers with overlapping cell migration and survival programmes that, decades earlier, secured implantation, shaping and survival of the embryo.

Combined impact on outcome

The signalling intersections between metastasis and the development of therapy resistance have been identified and validated using controlled 3D tissue culture and preclinical animal models. By comparison, analyses correlating molecular signatures in clinical samples with therapy response and outcomes in patient cohorts have delivered only partial conclusive support for coordinated metastasis signalling and therapy resistance signalling contributing to poor outcomes[166]. Pooled results from multiple trials of cardiovascular prophylaxis indicate that daily therapy with aspirin, which modulates the inflammatory response in neutrophils and macrophages[167], reduces the frequency of distant metastasis and deaths in certain epithelial cancers that were non-metastatic at diagnosis[168]. These patient cohorts included cytostatic treatment groups, suggesting that lipid mediator signalling and inflammatory damage caused by chemotherapy could be reduced by aspirin, with the consequence of decreasing the risk of metastatic progression. Similar circumstantial evidence supports the notion that chemotherapy and radiotherapy may, in at least some cases, increase the risk of metastatic cancer progression[169]. Subgroups of patients with breast cancer develop increased CTC numbers and distant metastasis after neoadjuvant chemotherapy; here, metastasis is associated with incomplete therapy response followed by residual disease, whereas patients achieving a complete response lack an increased

risk of metastasis[170]. These data indicate that resistant cells survive chemotherapy and retain the ability to metastasize. In clinical tumour specimens, invading epithelial cancers show a prominent collective invasion pattern, in which EMT markers, including ZEB1 and TWIST, are upregulated[160]. The extent of collective invasion is correlated with accelerated metastasis in cohorts of patients who have undergone chemotherapy and radiotherapy[171]. However, no clinical data are available linking the presence of EMT-positive invasion zones to preferential resistance to chemotherapy or radiotherapy and consequently accelerated metastasis.

Conversely, clinical data suggest that the therapy stress does not directly induce therapy resistance and metastatic escape, at least not in most tumour cells. Hormonal therapy, chemotherapy and radiotherapy reliably reduce the number of CTCs in responding patients, indicating a strong antimetastatic effect by, for example, reducing the overall tumour burden and the viability of the cells before they enter circulation[172–174]. However, whether the persisting subsets of CTCs that, albeit diminished in number, did survive therapy have acquired traits of therapy resistance and have gained a particular ability to initiate relapsing metastatic disease remains to be determined. The reasons for inconclusive or discordant outcomes between preclinical and clinical analyses may relate to the transient nature of signalling adaptation and/or the development of combined metastasis and therapy resistance in comparably small but functionally significant cell subsets, the detection of which may fail when bulk population measurements are used and may instead require sensitive single-cell isolation and detection approaches, such as single-cell genomics and transcriptomics analyses.

Towards joint druggability

Signalling hubs involved in both metastasis and the development of therapy resistance are attractive therapeutic targets for inhibiting both processes in concert. Shared pathways include PI3K–AKT–mTORC, MAPK–JNK, FAK–SRC, the RHOA-mediated actomyosin contractility and integrin signalling (FIG. 5a,b), and strategies to target these pathways have been explored both in vitro, in 3D growth and invasion cultures, and in preclinical studies with tumour cell survival, therapy resistance development or metastatic growth as end points[101,104,125,175–178] (see Supplementary Table 1). Similarly, inhibitors targeting stress response pathways, including HIFs, mTORC, survivin, ESCRTIII and RHOA-mediated actomyosin contractility have been developed, in part for non-oncological indications (FIG. 5c). Context-independent target pathways originating from inside the tumour cell, including oncogenic signalling, or endocrine stimulation, such as androgen or oestrogen stimulation, provide additional signalling in metastatic cells, irrespective of the actual organ site, and can be effectively inhibited by oncogenic and hormonal pathway inhibitors, respectively (FIG. 5d). As an orthogonal strategy, inhibition of effectors of the DNA damage response, including CHK1, CHK2 and PARP1, and enzymes mediating epigenetic reprogramming, such as HDACs and HMTs, have been developed (FIG. 5). Because of the central role of HDACs in epigenetic reprogramming of cancer cells, selective HDAC inhibitors have been developed to interfere with multipathway programmes involved in metastasis and therapy resistance[179]. As an example, HDAC inhibitors inhibit epithelial cancer invasion and growth

programmes by interfering with CRK-like protein (CRKL), a multifunctional adapter protein connecting integrin adhesion and tyrosine kinase signalling[180].

Importantly, drugs interfering with individual pathways may have failed in clinical trials involving patients with late-stage cancer using conventional end points as criteria (that is, progression-free or overall survival); however, when repurposed and administered in tailored combination schemes in selected patient subsets, these compounds may be effective in eliminating metastatic cells that have undergone therapy resistance. For instance, anti-inflammatory treatment may show potential to be combined with conventional therapy and reduce the risk of both therapy resistance and metastasis. Here, the inflammatory response caused by the chemotherapeutics vincristine and doxorubicin upregulates expression of IL-1β, IL-6, and CXCL1 in the TME, which can be effectively mitigated by combination with MAPK inhibition[181]. The suitability of each molecular pathway for combined targeting to prevent therapy resistance in neoadjuvant and adjuvant therapy settings remains to be explored. Furthermore, as end points, the ability to eliminate minimal residual disease (for example, detected by bone marrow aspiration) or metastatic dissemination (detected as the number of CTCs in the peripheral blood) remain to be explored for tumours of different origin, stage, mode of metastasis (that is, individual or collective) and therapy type. However, there is promise as modulation of both metabolic and epigenetic signals via the AMPK–SETD2–enhancer of zeste homologue 2 (EZH2) axis by administration of metformin has been demonstrated to suppress both epithelial cancer metastasis[182] and resistance to chemotherapy and radiotherapy[183]. By exploiting these and additional intervention points (see Supplementary Table 1), strategies towards multitargeted therapies will depend on defining and validating shared hubs of invasion and survival programmes.

Conclusions and perspectives

Converging experimental evidence is indicating that tumour cell metastasis and survival programmes support each other reciprocally. Mechanistic explanations for this are beginning to be revealed, residing largely to date in complex signalling pathway crosstalk and integrative governance of diverse downstream effector processes. Future research in dissecting metastasis and therapy resistance crosstalk mechanisms in vivo, including in patients, will require identification of shared critical pathways involved in the stepwise reprogramming of transformed cells through a metastatic cascade — the so-called Achilles heel, the targeting of which may induce a dual benefit in abrogating both tumour cell survival and tumour cell dissemination. Whereas the crosstalk between invasion and metastasis and therapy resistance signalling has been reported for rapidly growing solid tumours, it is unclear how this concept applies to non-proliferating, dormant tumour cells and haematopoietic cancers. Dormant cells are drug tolerant and can withstand cytotoxic and molecular therapies; however, they are considered as organotropic and largely non-migrating. Hence, very different mechanisms of therapy resistance may be at work. By contrast, leukaemias and lymphomas are composed of constitutively motile cells; therefore, abundant cytoskeletal activity and promigratory signaling can be expected to contribute to survival pathways.

The hurdles in ascertaining these kinds of shared targets are severalfold. First, signalling crosstalk and redundancies identified in preclinical models (FIG. 4) may preclude single-target approaches, and instead rational combination treatments using multitargeted intervention may be required. As an example, dual-integrin targeting, but not single-integrin targeting, provides superior radiosensitization and mitigates metastatic escape in preclinical cancer models[11]. Second, the heterogeneity and plasticity of the signals present in the TME limit the efficacy of targeting a single pathway or effector mechanism. This includes the effects of disrupted negative feedback loops and the activation of compensatory pathways, such as with AXL-mediated survival signalling[184]. Understanding how complementary processes cooperate, such as the stress response, migration-enhancing cytokine signalling and immune evasion, may help to identify orthogonal interventions to combat both instantaneous and long-lasting coping strategies used by cancer cells. Third, in contrast to traditional targeted therapy strategies, for combination treatments we lack clinically applicable biomarkers for the selection of patients who may be at risk of acquired resistance development and are vulnerable to metastatic evasion, and for the assessment of their anticancer effects. Fourth, cells under pressure to metastasize or exposed to chemotherapy and radiotherapy may experience opposing effects, and vulnerability may be enhanced by additive challenge. As an example, cell debris and upregulation of immune epitopes in response to drug treatment may increase the antigenicity of tumours and improve immunotherapy[185]. Similarly, cancer cells moving in interstitial tissue may be more exposed to immune cells and become detected by cytotoxic effector cells and eliminated[186]. Predicting the outcome of agonistic and antagonistic signals and their relevance for metastatic and survival end points will require multivariate systems approaches integrating molecular and cellular profiling with computational modelling to determine the quantitative balances governing signal–outcome relationships[187,188]. Additionally, new invasion-associated targeting principles will have to be defined. For example, cell–cell adhesion between tumour cells supports both effective distant organ colonization and cancer cell survival by providing mechanical protection and effective paracrine growth factor signalling and dampening cell damage by ROS respiratory stress[12,22,189]. Thus, disrupting cell–cell adhesion and juxtacrine signalling mechanisms between tumour cells themselves and between tumour cells and stromal cells (BOX 1) may enable novel avenues to prevent both collective mechanisms of metastasis and therapy resistance.

Developing paradigms for combined treatments targeting metastasis and therapy resistance will require optimized pharmaceutical regimens and (pre)clinical trial designs. The adaptive complexity of the communication network within the TME and the effect on resistance of tumour growth is becoming increasingly appreciated by use of single-cell RNA sequencing and genomic analyses, which is critical to identify non-redundant pathways for targeted treatment strategies[8,187]. Thus, integrative high-resolution analyses using multi-omics, single-cell technologies and/or spatially based technologies are required to be integrated in clinical trial designs; this will provide deeper insights into local therapeutic responses and the exact cellular and molecular consequences of combined metastasis and therapy resistance initiation and propagation, and biomarkers suited to the identification of patient subsets that would benefit. To this end, the deployment of personalized multi-agent regimens and computational benchmarking will define predictive paths to metastasis and

therapy resistance prevention in high-risk patients, providing workflows bridging molecular modelling of networks[190,191]. Because many of these sophisticated techniques are typically performed in mouse models, a principled framework for translating data-driven inferences from mouse to human contexts will be needed[191]. This will provide a personalized medicine perspective to identify the best-suited molecular strategies to inhibit cell-intrinsic and context-dependent signalling in patient subsets. To achieve this, predictive histology, genomic alterations or clinical staging may be necessary to indicate patients with a high risk of metastasis or a high burden of CTCs or ongoing metastatic tumour cell circulation. Considering the advances outlined herein, researchers and oncologists working together should advance the identification and validation of successful strategies to inhibit or prevent the crosstalk supporting therapy resistance and metastatic progression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Adaptive resistance
Resistance resulting from stress programmes induced in cancer cells by a range of external triggers, including therapy stress, metabolic perturbation, and cytokine-mediated stemness or epithelial-to-mesenchymal transition.

Anoikis
A form of programmed cell death of anchorage-dependent cells that is activated upon detachment from the extracellular matrix due to the lack of growth and survival signals provided from the matrix interaction.

Autophagy
A controlled pathway in which autophagosomes engulf and degrade cellular organelles as an alternative source for energy production and cell survival.

Cell detritus
Interstitial cell fragments, including cell membranes, organelles and DNA.

Chromatin organization
The 3D structure of DNA, under the control of histone proteins. The density of chromatin packaging determines the accessibility of the genome to transcription factors.

Context receptors

A heterogeneous group of cell surface receptors that provide intracellular signals in response to binding extracellular matrix, matrix-associated growth factors and adjacent cell-surface receptors.

DNA methyltransferases
(DNMTs). A group of enzymes that introduce methylation of cytosine and guanine-rich regions of the DNA and repress transcription by recruitment of methyl-CpG-binding proteins.

Endosomal sorting complexes required for transport III
(EsCRTIII). A complex of cytosolic proteins forming a machinery able to remodel and repair cell membranes.

Epithelial-to-mesenchymal transition
(EMT). The conversion of polarized, adherent epithelial cells into motile mesenchymal cells that lack apicobasal polarity and possess decreased cell–cell adhesion strength and acquire stem cell-like traits.

Hippo pathway
A mechanosensitive pathway that controls cell size, division and apoptosis. In morphogenesis, Hippo pathway activation limits growth and induces apoptosis, whereas in cancer cells it enhances oncogenic signalling.

Histone acetyltransferases
(HATs). A group of enzymes that add acetyl groups to the histone tail; this weakens the strength of binding to DNA, reduces chromatin density and facilitates access of transcription factors to DNA.

Histone deacetylases
(HDACs). A group of enzymes that remove acetyl groups from the histone tail; this strengthens the histone–DNA interaction, leads to chromatin condensation and reduces transcription.

Histone demethylases
(HDMs). A group of enzymes that remove methyl groups from the histone tail, which reduces chromatin density.

Histone methyltransferases
(HMTs). A group of enzymes that add methyl groups to the histone tail, which favours heterochromatization by recruitment of chromatin-binding proteins, which increases chromatin density, decreases DNA accessibility and silences transcription.

Histone-modifying enzymes
Enzymes that induce reversible acetylation and methylation of histones, which regulates the chromatin structure and density, and thereby the local accessibility of DNA for transcription factors and DNA damage response proteins.

Integrin

Adhesion receptor, which engages with extracellular matrix and other ligands and mechanically connects to the actin cytoskeleton for cell anchorage and migration.

Lipid mediators
Metabolites of polyunsaturated fatty acids, including leukotrienes and prostaglandins, which are acutely released by leukocytes to induce and regulate local inflammation.

Matrix metalloproteinases
(MMPs). A large family of proteolytic secreted or membrane-bound enzymes that degrade a broad range of substrates, including extracellular matrix, growth factors and surface receptors.

Nanolumenal release
Extracellular secretion of vesicle content into very tight spaces between cell–cell junctions, which limits dilution of released cytokines and enables particularly strong autocrine and juxtacrine signalling.

Senescence
A cellular state of sustained growth arrest in response to stress. It is associated with increased resistance to cell death.

Shear stress
The physical force exerted on circulating tumour cells by blood flow.

Survivin
Belongs to the inhibitor of apoptosis (IAP) protein family, which inhibits caspases and thereby suppresses apoptosis.

Tissue inhibitor of metalloproteinases 3
(TIMP3). An important broad-spectrum inhibitor of matrix metalloproteinases produced by tumour and stromal cells that inhibits epithelial-to-mesenchymal transition and metastatic progression.

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Box 1 |

Signalling differences between single-cell invasion and collective invasion

Systemic cancer can be mediated by single-cell invasion or collective invasion and metastasis[12], which differ in morphology, signalling pathways and survival mechanisms. Individual cancer cell migration engages cell–matrix and microenvironmental signals, but typically lacks signals provided by cell–cell interactions that are present during collective invasion and metastasis. Both migration modes depend on (1) cell-intrinsic signalling networks required to steer the migration machinery and (2) signals from the extracellular matrix, heterotypic interactions between tumour and stromal cells, and cytokine signalling from the tumour stroma. Collective invasion, but not single-cell invasion, additionally (1) maintains homotypic cell–cell adhesion signalling and (2) is associated with increased autocrine and paracrine signalling[28], as well as strategies to overcome mechanical, chemical and metabolic challenges by virtue of the homotypic cell interactions[22,23,189]. Even during the process of epithelial-to-mesenchymal transition (EMT), cell–cell adhesions are often partially and only rarely completely downregulated, and so a moderate level of cell–cell interactions during collective metastasis enables cancer cells to move through tissues with a strongly increased survival ability[12,192]. Adherens junctions mediate prosurvival signalling through cyclin-dependent kinase (CDK) inhibitor p27-mediated PI3K–AKT, RAS and RAC1 signalling, MAPK signalling and Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) signalling[193,194]. Moreover, the adaptable, cooperative signalling networks that arise from adherens junction engagement may contribute to the increased efficiency (as much as 50-fold) of collective cancer cell groups and clusters to invade and establish distant metastases[19,20,22,26]. Which mode of invasion dominates in different cancer types is currently being evaluated in preclinical and clinical investigations.

Box 2 |

Cell interactions beyond cadherins and integrins

Contacts between tumour cells

Besides E-cadherin, a number of additional cell–cell adhesion systems mediate cell–cell interaction and collective processes, including tumour cell survival and metastasis. Desmosomal cadherins connect to intermediate filaments via plakoglobin, provide mechanically stable connections and protect circulating tumour cells from death in the bloodstream[22]. Arguably weaker cell–cell interactions result from homophilic cell adhesion molecules of the immunoglobulin superfamily, including neural cell adhesion molecule (NCAM), activated leukocyte cell adhesion molecule (ALCAM) and neural cell adhesion molecule L1 (L1CAM), as well as CD44, which cooperate with integrins and activate both MAPK signalling and the small GTPases RAC1 and RHOA[195,196]. In mesenchymal tumours and glioma, which lack E-cadherin expression, other cadherins and immunoglobulin superfamily cell adhesion molecules as well as connexins are expressed and contribute to tissue invasion[189,197].

Tumour cell–extracellular matrix contacts

Likewise, a range of non-integrin cell–extracellular matrix interactions have been implicated in mediating shared invasion and survival programmes. CD44 is a multifunctional adhesion receptor that interacts with extracellular hyaluronic acid and multiple other ligands, including CD44 itself, osteopontin (OPN) and matrix metalloproteinases (MMPs)[195,198]. CD44 recruits ezrin, radixin and moesin (ERM) proteins by its intracellular tail[199] to bind actin and activate Rho GTPases and PI3K–AKT[200] (FIG. 1c). CD44 further mediates cell–cell interactions during collective metastasis[195]. Discoidin domain receptors (DDRs) are receptor tyrosine kinases (RTKs) that interact with collagen, which can stimulate migration and metastasis, albeit not through direct interactions with the actin cytoskeleton[201]. DDRs activate the SRC and ERK pathways as well as SNAI1, inducing epithelial-to-mesenchymal transition (EMT) programmes, and thereby promote invasion and metastasis, in cooperation with integrins[202].

Heterotypic contacts with stromal cells

Invading tumour cells can mechanically interact with stromal fibroblasts and/or macrophages, through cadherins, which supports invasion of basement membrane and interstitial tissue[203,204] (FIG. 1a). Tumour cell–stromal cell clusters can further jointly spread into the circulation and metastasize[204–206]. Other heterotypic contacts, mediated by integrins, include adhesion to endothelial cells of blood vessels during extravasation or to macrophages at the metastatic site[207]. These cell–cell interactions connect to the actin cytoskeleton, and contribute to cell anchorage to extracellular matrix, as well as migration and metastasis.

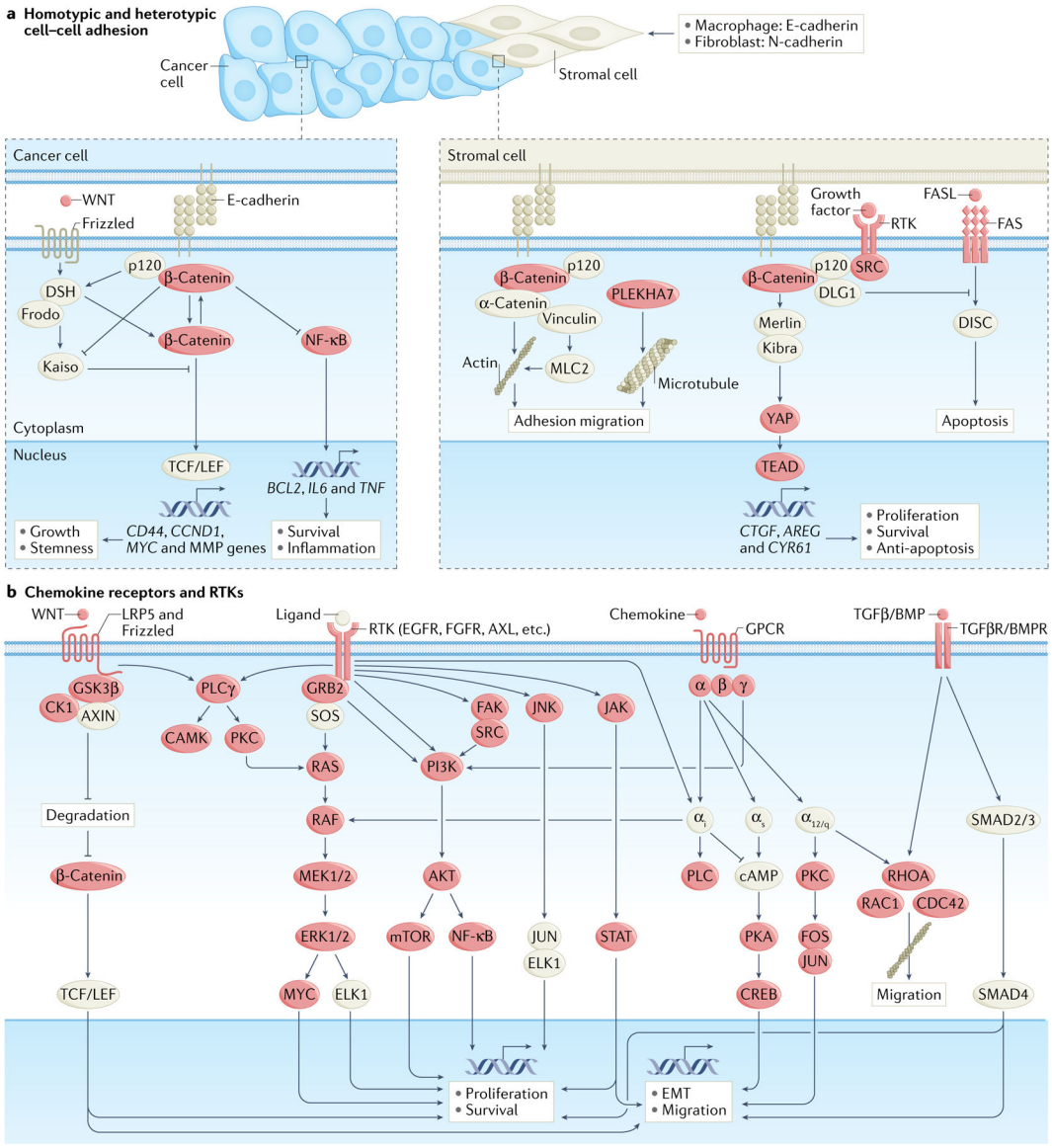

Fig. 1 |. Invasion-associated reprogramming.

Targets for selective inhibitors of soluble mediators, cell surface receptors, context receptors and kinases are coloured red. For these highlighted targets, often multiple classes of inhibitors have reached the preclinical or clinical trial stage. See online databases for chemical compounds at the Chemical Probes Portal and for targeting antibodies at the Therapeutic Structural Antibody Database. **a** | The upper schematic depicts homotypic interactions between cancer cells and heterotypic interactions of cancer cells with stromal cells during collective invasion. Upon homotypic interactions, engaged E-cadherin, p120–catenin and WNT–activated dishevelled (DSH) prevent β-catenin degradation, and p120 antagonizes negative feedback through Frodo and Kaiso. T cell factor (TCF)/lymphoid enhancer-binding factor (LEF) induce transcription of *CD44*, *MYC* and cyclin D1 (*CCND1*) and genes encoding matrix metalloproteinases (MMPs), promoting proliferation and stemness. E-cadherin engagement also limits nuclear factor-κB (NF-κB) signalling,

repressing expression of BCL-2, interleukin-6 (IL-6) and tumour necrosis factor (TNF) and thereby repressing apoptosis and inflammation. During heterotypic interactions, engaged E-cadherin interacts with α-catenin, vinculin, myosin light chain 2 (MLC2) and pleckstrin homology domain-containing A7 (PLEKHA7) to promote cell–cell adhesion and migration. p120 activates Merlin and Kibra, in turn activating Yes-associated protein (YAP) and TEA domain family member (TEAD) and inducing expression of connective tissue growth factor (*CTGF*; also known as *CCN2*), amphiregulin (*AREG*) and cysteine-rich angiogenic inducer 61 (*CYR61*; also known as *CCN1*), which leads to induction of proliferation and survival and inhibition of apoptosis. Interaction of cadherins with receptor tyrosine kinases (RTKs), through SRC activates Discs large homologue 1 (DLG1) blocking the FAS cell surface death receptor-mediated activation of death-inducing signalling complex (DISC) and repressing apoptosis. **b** | The signalling pathways downstream of ligation of RTKs and G protein-coupled receptors (GPCRs), and the pathway of stromal signalling caused by growth factors and extracellular matrix molecules released by stromal cells, including fibroblasts, macrophages and endothelial cells. Ligand binding induces RTK dimerization and downstream activation of phospholipase Cγ (PLCγ), which in turn activates calcium/calmodulin-dependent protein kinase (CAMK) and protein kinase C (PKC). Growth factor receptor-bound protein 2 (GRB2) and Son of Sevenless (SOS) activate PI3K–AKT — mTOR complex (mTORC) and RAS–RAF–MEK1/MEK2–ERK1/ERK2–MYC/ELK signalling. PKC activates RAS and MAPK pathways. PKC further activates FOS and JUN. Janus kinase (JAK) activates signal transducer and activator of transcription (STAT). Chemokines activate GPCRs, and downstream Gα subunits dissociate from the G α–β–γ complex and activate further pathways: Gα inhibitory ($G\alpha_i$) activates PLC and MAPK, and inhibits cAMP; Gα stimulatory ($G\alpha_s$) activates cAMP and downstream protein kinase A (PKA) and cAMP responsive element-binding protein (CREB). Other Gα isoforms activate PKC, RHOA, RAC1 and CDC42, which induce actin dynamics. The G β–γ dimer activates PI3K signalling. As well as GPCRs, RTKs also activate $G\alpha_i$. Transforming growth factor-β (TGFβ) receptor (TGFβR) activates RHOA and Rho-associated protein kinase (ROCK), which regulate cytoskeletal organization. TGFBR and bone morphogenetic protein (BMP) receptor (BMPR) activate SMAD family members and TCF/LEF-mediated transcription, leading to proliferation and survival. WNT, through engagement of Frizzled and low-density lipoprotein receptor-related protein 5 (LRP5), recruits casein kinase 1 (CK1), glycogen synthase kinase 3β (GSK3β) and AXIN, activating RHOA, β-catenin, PLC, PKC and downstream effectors (for example, nuclear factor of activated T cells (NFAT)), inducing proliferation and survival. EGFR, epidermal growth factor receptor; EMT, epithelial-to-mesenchymal transition; FAK, focal adhesion kinase; FASL, FAS ligand; FGFR, fibroblast growth factor receptor; JNK, JUN amino-terminal kinase.

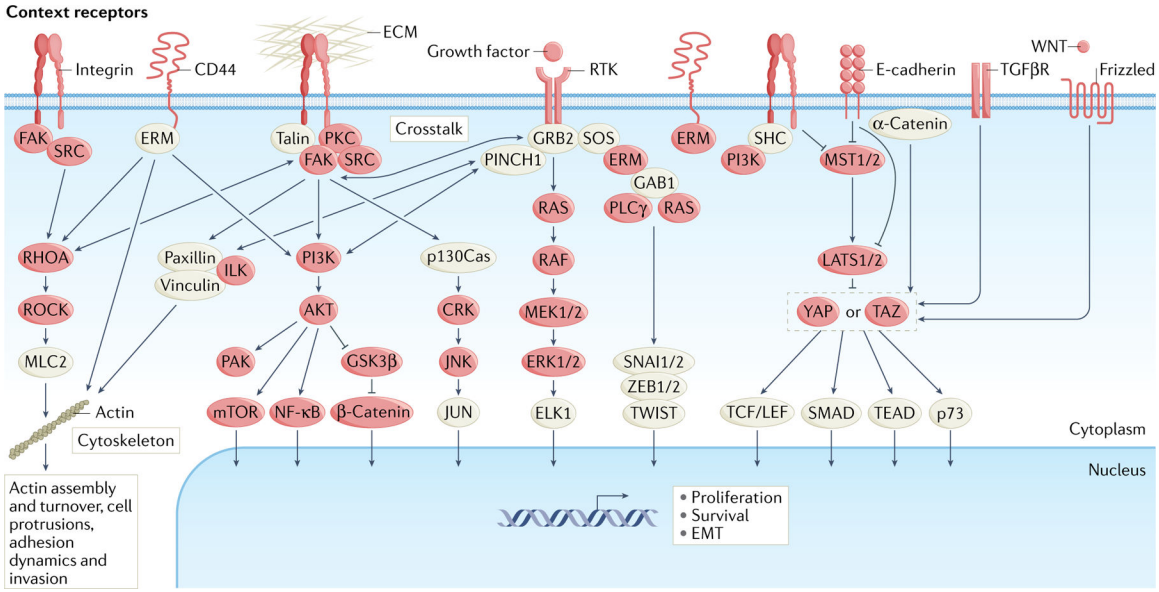

Fig. 2 |. Invasion-associated reprogramming from the extracellular matrix.

Targets for selective inhibitors of soluble mediators, cell surface receptors, context receptors and kinases are coloured red. For these highlighted targets, often multiple classes of inhibitors have reached the preclinical or clinical trial stage. See online databases for chemical compounds at the Chemical Probes Portal and for targeting antibodies at the Therapeutic Structural Antibody Database. Integrin receptors recruit the adapter talin and subsequently activate focal adhesion kinase (FAK) and SRC. Downstream of this, RAC1, CDC42, RHOA, Rho-associated protein kinase (ROCK) and myosin light chain 2 (MLC2) mediate cytoskeletal organization and migration. FAK activates paxillin, vinculin, integrin-linked kinase (ILK) and PI3K, activating AKT and β-catenin. FAK also activates the CRK-associated substrate p130Cas and downstream CRK, JUN amino-terminal kinase (JNK) and JUN. Crosstalk between receptor tyrosine kinase (RTK) and focal adhesion signalling through growth factor receptor-bound protein 2 (GRB2), Son of Sevenless (SOS) and particularly interesting new Cys-His protein 1 (PINCH1) activates MAPK pathways. CD44 in conjunction with RTKs (for example, MET) and integrins recruits ezrin, radixin and moesin (ERM) proteins, GRB2, GRB2-associated-binding protein 1 (GAB1), RAS, SHC and PI3K, which in turn promotes recruitment of phospholipase Cγ (PLCγ), SNAI1, SNAI2, ZEB1, ZEB2 and TWIST, which leads to induction of epithelial-to-mesenchymal transition (EMT). WNT and transforming growth factor-β (TGFβ) both activate Yes-associated protein (YAP). Integrins and E-cadherin inhibit mammalian STE20-like protein kinase 1 (MST1) and MST2, repressing large tumour suppressor homologue 1 (LATS1) and LATS2 activation and thereby derepressing YAP signalling. E-cadherin activates YAP directly through α-catenin. YAP co-activates T cell factor (TCF)/lymphoid enhancer-binding factor (LEF), SMAD, TEA domain family member (TEAD) and p73, inducing transcription of connective tissue growth factor (*CTGF*), integrin β2 (*ITGB2*), fibroblast growth factor 1 (*FGF1*), *MYC*, baculoviral IAP repeat-containing protein 2 (*BIRC2*), baculoviral IAP repeat-containing protein 5 (*BIRC5*) and p53 upregulated modulator of apoptosis (*PUMA*; also known as *BBC3*), in turn promoting proliferation and inhibition of apoptosis. ECM, extracellular matrix; GSK3β, glycogen synthase kinase 3β; NF-κB, nuclear factor-κB;

PAK, p21-activated kinase; PKC, protein kinase C; TGFβR, transforming growth factor-β receptor.

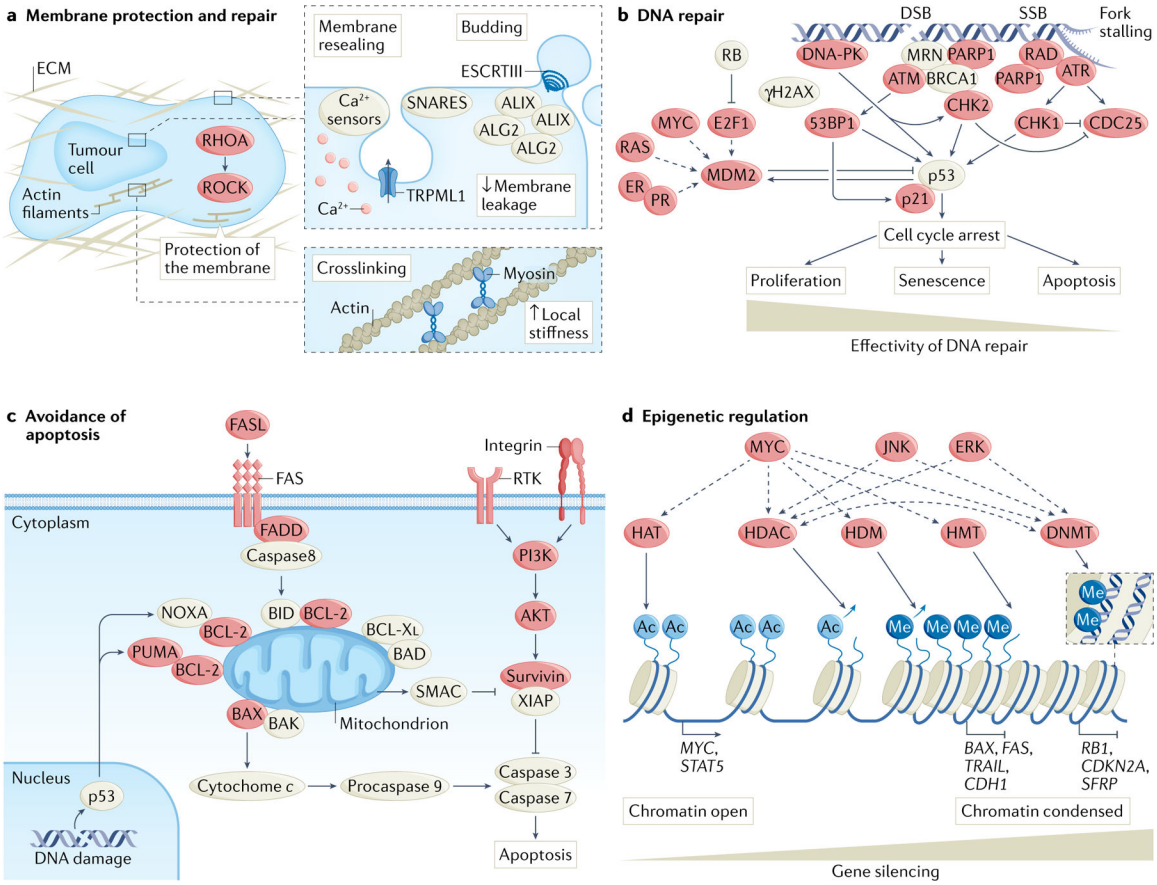

Fig. 3 |. Mechanisms of repair and cell survival.

Signalling pathways associated with membrane and DNA repair, avoidance of apoptosis and epigenetic mechanisms, all of which secure cell survival and have been shown to mediate therapy resistance, individually or through cooperation. Receptors and signalling molecules marked in red can be inhibited pharmacologically. **a** | Membrane repair and protection. Membrane defects are repaired by recruitment of Ca^{2+} sensors, transient receptor potential channel mucolipin 1 (TRPML1)-mediated export of Ca^{2+} and SNARE proteins. The damaged site undergoes budding and resealing through the recruitment of endosomal sorting complexes required for transport III (ESCRTIII), ALG2-interacting protein X (ALIX) and asparagine-linked glycosylation protein 2 homologue (ALG2). Membrane stress is prevented by RHOA–Rho-associated protein kinase (ROCK)–myosin light chain 2 (MLC2)-mediated crosslinking of F-actin and cortical stiffening. **b** | DNA repair. Different forms of DNA damage trigger the activation of specific repair systems, including homologous recombination (HR) and non-homologous end joining (NHEJ), among other repair mechanisms. Double-strand breaks (DSBs) are repaired by DNA-dependent protein kinase (DNA-PK), poly(ADP-ribose) polymerase 1 (PARP1) and the MRE11, RAD50 and nibrin (MRN) complex, which recruit ataxia telangiectasia mutated (ATM) and BRCA1. Together, the multiprotein complex that forms modulates the activity of p53 and p21, directly or indirectly through the histone variant γH2AX, p53-binding protein 1 (53BP1) or checkpoint kinase 2 (CHK2). Single-strand breaks (SSBs) are recognized by several RAD proteins, which engage ataxia telangiectasia and Rad3-related protein (ATR) and

activate CHK1 and p53. CHK1 and CHK2 cause cell cycle arrest through inhibition of cell division cycle 25 (CDC25). Oncogenic and hormonal activation of MDM2, which inhibits p53, counteracts cell cycle arrest. **c** | Avoidance of apoptosis. The intrinsic death pathway is initiated by cellular stress. DNA damage activates p53, which induces transcription of the proapoptotic genes *NOXA*, p53 upregulated modulator of apoptosis (*PUMA*), BH3-interacting domain death agonist (*BID*), BCL-2 associated agonist of cell death (*BAD*), BCL-2 antagonist/killer (*BAK*) and *BAX* and the anti-apoptotic genes *BCL2* and *BCLX*, the protein products of which activate cytochrome *c*, procaspase 9, caspase 3 and caspase 7. FAS ligand (FASL)–FAS, FAS-associated via death domain (FADD) and procaspase 8 activate the extrinsic death receptor pathway. Second mitochondria-derived activator of caspase (SMAC; also known as DIABLO) inhibits anti-apoptotic survivin (also known as BIRC5) and X-linked inhibitor of apoptosis (XIAP). **d** | Epigenetic regulation. Oncogenic signalling through MYC, JUN amino-terminal kinase (JNK) and ERK regulates histone-modifying enzymes. Histone acetyltransferases (HATs) add acetyl (Ac) groups to histones, which opens the chromatin structure and upregulates expression of oncogenes (for example, MYC and signal transducer and activator of transcription 5 (STAT5)); this can be reversed by histone deacetyltransferases (HDACs). Histone methyltransferases (HMTs) condense the chromatin, which represses expression of tumour suppressors, such as the genes encoding BAX, FAS, tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) and E-cadherin; this can be reversed by `histone demethylases` (HDMs). DNA methyltransferases (DNMTs) methylate DNA, which represses the expression of tumour suppressors, including the genes encoding RB, cyclin-dependent kinase (CDK) inhibitor 2A (CDKN2A) and secreted Frizzled-related protein (SFRP). ECM, extracellular matrix; ER, oestrogen receptor; Me, methyl; PR, progesterone receptor; RTK, receptor tyrosine kinase.

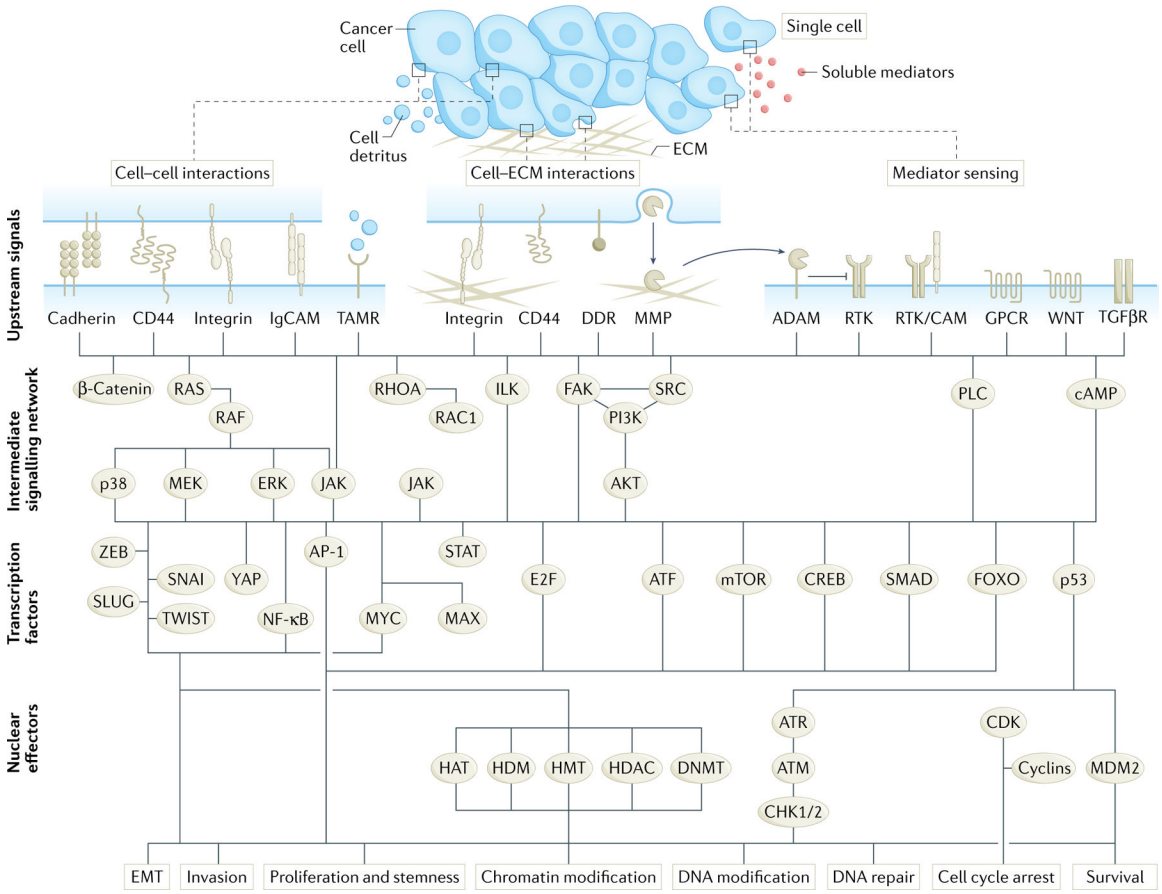

Fig. 4 |. Cooperation and redundancy of survival signalling in single-cell and collective invasion during metastasis.

Multiple signalling pathways cooperate to ensure survival in invading single cells, including signalling pathways downstream of context receptors and cytokines and, additionally, in collectively invading cells, signalling pathways downstream of cell–cell adhesions. These pathways not only maintain cell–cell cooperation and collective invasion but also through crosstalk enable the development of therapy resistance. Some pathways and transcription factors such as E2F more strongly affect survival and proliferation, whereas other pathways are more related to invasion. However, most pathways are interconnected and therefore affect all key cell functions. ADAM, a disintegrin and metalloproteinase; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related protein; CAM, cell adhesion molecule; CDK, cyclin-dependent kinase; CHK, check-point kinase; CREB, cAMP responsive element-binding protein; DDR, discoidin domain receptor; DNMT, DNA methyltransferase; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; FAK, focal adhesion kinase; FOXO, forkhead box O; GPCR, G protein-coupled receptor; HAT, histone acetyltransferase; HDAC, histone deacetyltransferase; HDM, histone demethylase; HMT, histone methyltransferase; IgCAM, immunoglobulin superfamily cell adhesion molecule; ILK, integrin-linked kinase; JAK, Janus kinase; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB; PLC, phospholipase C; RTK, receptor tyrosine kinase; STAT, signal transducer and activator of transcription; TAMR, TYRO3,

AXL and MERTK family receptor; TGFβR, transforming growth factor-β receptor; YAP, Yes-associated protein.

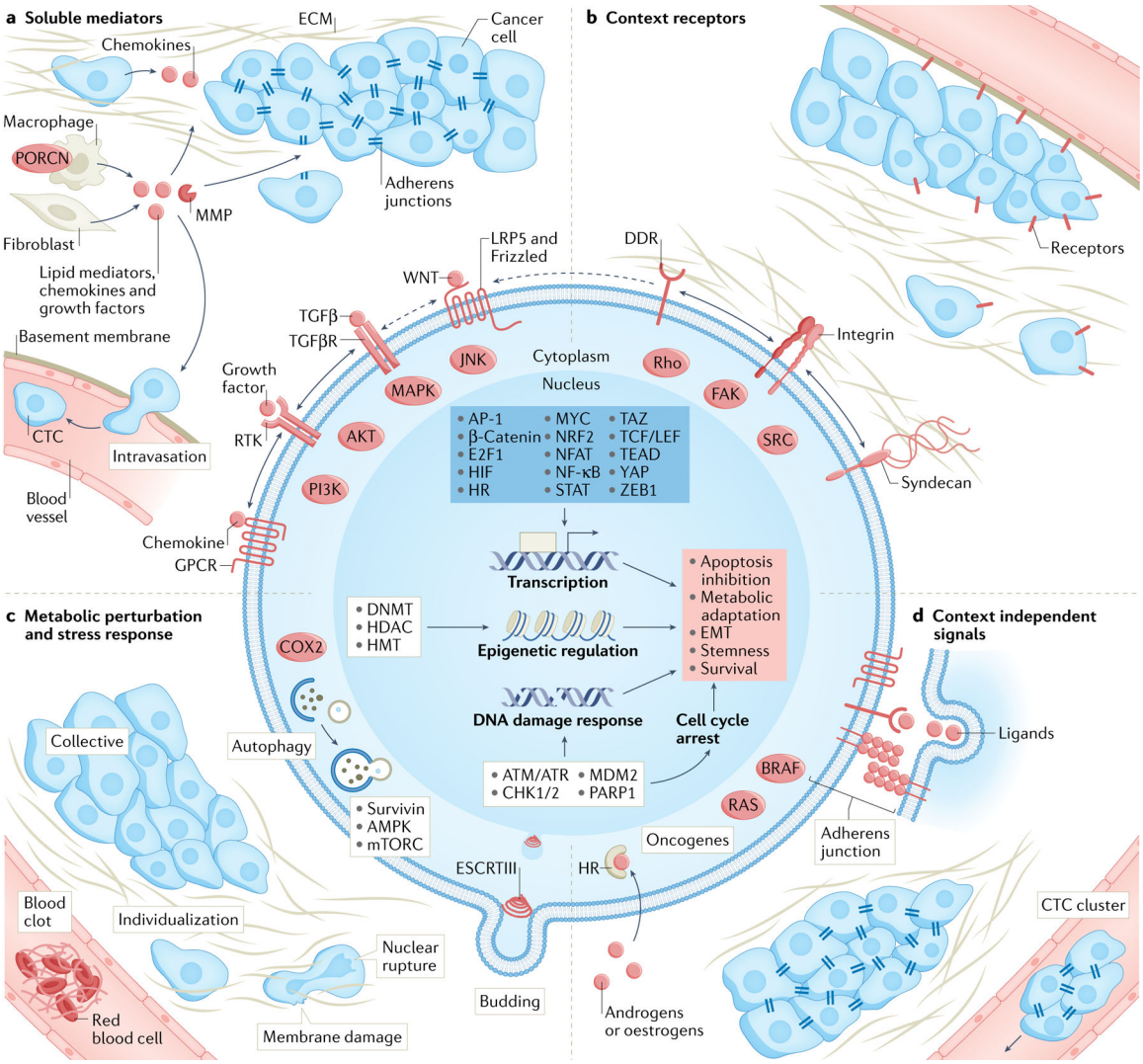

Fig. 5 |. Targeting metastasis-associated therapy resistance programmes.

This schematic shows the coexistence of signalling pathways and cell functions as well as the intervention points to interfere with both invasion and metastatic evasion and therapy resistance of cancer cells. Targets for selective inhibitors of soluble mediators, cell surface receptors, context receptors, kinases and other regulators are coloured red or listed in white boxes and transcription factors are coloured blue. For these highlighted targets, often multiple classes of inhibitors have been developed that have reached the preclinical or clinical trial stage, but are not listed here explicitly. See online databases for chemical compounds at the Chemical Probes Portal and for targeting antibodies at the Therapeutic Structural Antibody Database. **a** | Soluble mediators from the reactive tumour microenvironment, including growth factors, chemokines, lipid mediators and matrix metalloproteinases (MMPs), directly or indirectly activate collective and single-cell invasion and metastasis. **b** | Context receptors interact with interstitial extracellular matrix (ECM) and basement membrane. **c** | Stress responses result in metabolic reprogramming and adaptation of the mode of metastasis, from collective invasion to single-cell dissemination[91]. As an example, acute thrombosis of a peritumour microvessel results in

transient malperfusion, hypoxia and acidosis. **d** | Context-independent signalling, including adhesion signalling arising from interacting tumour cells, and hormonal signalling both provide survival signals independently of the local environment. AMPK, AMP-activated protein kinase; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related protein; CHK, checkpoint kinase; COX2, cyclooxygenase 2; CTC, circulating tumour cell; DDR, discoidin domain receptor; DNMT, DNA methyltransferase; EMT, epithelial-to-mesenchymal transition; ESCRTIII, endosomal sorting complexes required for transport III; FAK, focal adhesion kinase; GPCR, G protein-coupled receptor; HDAC, histone deacetyltransferase; HIF, hypoxia-inducible factor; HMT, histone methyltransferase; HR, hormone receptor; JNK, JUN amino-terminal kinase; LRP5, low-density lipoprotein receptor-related protein 5; mTORC, mTOR complex; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor-κB; NRF2, NFE2-related factor 2; PARP1, poly(ADP-ribose) polymerase 1; PORCN, porcupine; RTK, receptor tyrosine kinase; STAT, signal transducer and activator of transcription; TAZ, transcriptional co-activator with PDZ-binding motif; TCF/LEF, T cell factor/lymphoid enhancer-binding factor; TEAD, TEA domain family member; TGFβ, transforming growth factor-β; TGFβR, TGFβ receptor; YAP, Yes-associated protein.