Mutational drivers of cancer cell migration and invasion

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Genomic instability and mutations underlie the hallmarks of cancer—genetic alterations determine cancer cell fate by affecting cell proliferation, apoptosis and immune response, and increasing data show that mutations are involved in metastasis, a crucial event in cancer progression and a life-threatening problem in cancer patients. Invasion is the first step in the metastatic cascade, when tumour cells acquire the ability to move, penetrate into the surrounding tissue and enter lymphatic and blood vessels in order to disseminate. A role for genetic alterations in invasion is not universally accepted, with sceptics arguing that cellular motility is related only to external factors such as hypoxia, chemoattractants and the rigidity of the extracellular matrix. However, increasing evidence shows that mutations might trigger and accelerate the migration and invasion of different types of cancer cells. In this review, we summarise data from published literature on the effect of chromosomal instability and genetic mutations on cancer cell migration and invasion.

BACKGROUND
Genetic abnormalities lie at the heart of most cancers—mutations can transform normal cells into cancerous ones by endowing them with new properties. Genome instability and mutations determine the hallmarks of cancer, one of which is the ability of tumour cells to invade and metastasise.1 Metastasis is the leading cause of death from cancer. During the process of metastasis, tumour cells leave the primary site and spread throughout the body, forming secondary sites and causing severe organ failure.2 The first step of the metastatic cascade is invasion, in which tumour cells penetrate their surrounding basement membrane and migrate through the extracellular matrix (ECM) into the surrounding tissue (Fig. 1).3

Several different parameters in the tumour microenvironment influence the regulation of cancer cell migration and invasion: the presence of hypoxia, chemoattractants, ECM stiffness and a lack of nutrients prompt cancer cells to start searching for a 'better life'.4 Of particular significance during migration and invasion is the phenomenon of epithelial-to-mesenchymal transition (EMT), which determines the plasticity of tumour cells, allowing them to switch from a non-motile epithelial to a motile mesenchymal state, and endowing cancer cells with multiple malignant features, such as the increased invasiveness and resistance to senescence, apoptosis and treatment.4 The EMT is activated by transcription factors, such as Twist, Snail, Slug and Zeb1, through various signalling pathways, the most important being TGF-β, WNT and Notch pathways.5 The availability of these transcription factors can therefore offer a means of regulating this reversible and plastic process, with control also occurring at epigenetic and post-translational levels.5 The impact of somatic mutations incurred during primary tumour formation on EMT remains to be elucidated.2

The role of genetic alterations in tumour cell migration and invasion has received undeservedly little attention compared with epigenetic and transcriptional mechanisms of cell motility. Despite the huge amount of experimental data regarding the effect of genetic mutations on cancer invasion, only a few reviews exist, most of which focus mainly on the tumour suppressor p53.6,7 In this review, we summarise published data outlining chromosomal instability (CIN) and gene alterations that impinge on some of the molecular components that are crucial for cancer cell migration and invasion. We also discuss the main difficulties encountered in identifying genetic alterations that drive cancer invasion and suggest potential models and approaches to overcome these problems. Finally, we underscore the significance of identifying mutational drivers of cancer invasion as potential therapeutic targets for the prevention of metastatic disease.

CHROMOSOMAL INSTABILITY
CIN, which includes changes in the number of chromosomes as well as their rearrangement, is observed in many tumour types and is associated with tumour progression, as described in Box 1.8 For example, as shown in MDA-MB-231 triple-negative breast cancer cells in vitro and in vivo, CIN can induce the transcriptional transition of tumour cells to a mesenchymal state characterised by increased migratory and invasive behaviour with the activation of inflammatory pathways.9 By increasing inflammation, CIN can also promote cancer metastasis.9,10 It is worth noting, however, that CIN can influence the invasive and metastatic potential differently.

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CIN, one of the forms of genomic instability in tumours, is characterised by an increase in the rate of loss or gain of whole chromosomes or their fragments during cell division. CIN has a severe and complex impact on the genetic landscape of the tumour by affecting various oncogenes, tumour-suppressor genes and DNA-repair genes that drive cancer growth and progression. CIN promotes intratumoural heterogeneity and clonal evolution, giving cancer cells an advantage under selective pressure.

Different mitotic events underlie CIN. Among them are cohesion defects, dysfunction in spindle assembly checkpoint, centrosome amplification and cytokinesis failure. Defects in DNA replication and repair, such as telomere dysfunction and replication stress, are also responsible for CIN. All these changes lead to chromosome missegregation during mitosis and pave the way to polyploidy, aneuploidy and diverse chromosomal rearrangements.

The role of CIN in cancer growth and progression remains debatable. Some researchers consider CIN to be an early event in cancer, and some believe that CIN is simply a side effect of tumour growth. In any event, CIN is significantly associated with drug resistance and cancer progression.

Two types of CIN can be distinguished (Fig. 2): numerical CIN, which is determined by the gain or loss of whole chromosomes (aneuploidy) and chromosome sets (polyploidy), and structural CIN, which involves fractions of chromosomes and can result in gene fusions, amplifications and other alterations. In both cases, loss of heterozygosity (LOH)—defined as the loss of one allele caused by deletion, mitotic recombination, gene conversion or loss of a chromosome—can arise. LOH is a common alteration in cancer; it results in haploinsufficiency or loss of gene expression, and frequently affects tumour-suppressor genes, thereby contributing to tumorigenesis. In addition, LOH—alone or together with other genetic or epigenetic alterations—can influence the ability of cancer cells to invade. For example, LOH of the 8p22 chromosomal region (DLC1, which encodes a Rho GTPase-activating protein) promotes migration and invasion of breast, lung, prostate and liver cancer cells in vitro.

Amplification of genes and chromosomal regions (ErbB2, EGFR, 11q13, etc.)

Fig. 1 The model of cancer cell invasion. Cancer invasion is the first step of the metastatic cascade. Tumour cells penetrate the basement membrane and invade the surrounding tissues using two modes of movement—individual and collective invasion. Invading tumour cells reach the blood vessel, enter the blood flow and disseminate, eventually giving rise to secondary tumours.

Fig. 2 Chromosomal instability and cancer invasion. Chromosomal instability (CIN) is one of the cancer hallmarks and plays an important role in tumour cell migration and invasion. CIN can be represented by gain or loss of whole chromosomes (numerical CIN) and chromosomal rearrangements (structural CIN). Loss of heterozygosity (LOH) that can be attributed to numerical and structural CIN simultaneously, depending on the type of genomic changes resulting in the allele loss, affects the invasive potential of tumour cells. Polyploidy defined as the presence of additional sets of chromosomes drastically changes the genetic landscape of tumour cells, endowing them with high invasive potential. Polyploid giant cancer cells (PGCCs) are found in various cancers and show extreme tumorigenic, invasive and metastatic potential. Aneuploidy when chromosomes can be lost (monosomy) or gained (trisomy) can have different effects on tumour cell invasion: from attenuation of migratory behaviour to its enhancement. Different gene fusions arising from various chromosomal rearrangements affect tumour cell motility through diverse signalling pathways and mechanisms. Amplification defined as a copy number increase of a certain region of the genome leads to enhanced gene expression and, if a gene positively regulates cellular motility, it can accelerate cancer invasion.

region leads to changes in lipid metabolism, which, in turn, increases the motility and invasiveness of MCF10A breast cells in vitro. Loss of the expression of TGFBR3, which encodes TGF-β3, due to LOH of the 1p32 region, enhances migration and invasion of A549 non-small-cell lung cancer (NSCLC) cells in vitro.

Numerical CIN

Gain or loss of whole chromosomes (aneuploidy) or chromosome sets (polyploidy) are frequent events in various cancers and can drastically affect tumour progression not only through transcriptomic changes but also through the enhancement of CIN itself, creating more and more genetically distinct cancer cell clones.

It is believed that the polyploidisation of tumour cells is only a step on the path to aneuploidy. However, polyploid tumour cells can exist without transitioning to aneuploidy. Polyploid tumour cells contribute significantly to cancer progression. Polyploid giant cancer cells (PGCCs) are formed by endoreplication or fusion of several cells, and are found in high-grade and chemoresistant cancers, predominantly in breast, ovarian and colorectal cancers. PGCCs can survive anticancer
therapy, are extremely tumorigenic and contribute to cancer metastasis. PGCs and their daughter cells, collectively called tumour buds and located at the invasive front of tumours, have a mesenchymal phenotype and a high capacity for invasion through changes in the expression of factors that mediate EMT. In the MDA-MB-231 breast cancer cell line, PGCs moved more slowly than normal cancer cells, but showed high migratory persistence. This migratory phenotype is associated with the dysregulation of the actin network and RhoA–Rho-associated protein kinase (ROCK)1 signalling pathway, which drives increased cell stiffness. As shown in LoVo and HCT116 colorectal cancer cells in vitro and in vivo, the migration and invasion of PGCs and their daughter cells might be determined by S100A4 and its associated molecular network, potentially involving regulation of the structure and function of the annexin A2–S100A10 complex to influence cathepsin B, as well as cytoskeletal associations with 14–3–3 ζ/δ and ezrin. In addition to PGCs, other polyploid cells can contribute to tumour metastasis. For example, as shown in the DLD-1 cell line, tetraploid tumour cells observed at the invasive front of colorectal adenocarcinomas are characterised by an enhanced capability to migrate and invade.

Aneuploidy has long been known to be associated with an increased expression of genes related to EMT, cancer cell migration, invasion and metastasis. However, different aneuploidies have distinct effects on cancer cell invasion. For example, DLD-1 colorectal cancer cells with trisomy of chromosome 7 or 13 invade more actively than diploid cells, both in standard and stressful conditions (hypoxia, etc.) in vitro. Similarly, trisomy of chromosome 5 enhances the invasive potential of HCT116 colorectal cancer cells in vitro and in vivo through partial EMT and upregulation of matrix metalloproteinases (MMPs). By contrast, trisomy of chromosome 13 or 18 significantly decreases invasion of HCT116 colorectal cancer cells in vitro, potentially because of aneuploidy-induced dosage imbalances that may interfere with different cellular functions, including cell motility.

Structural CIN Chromosomal rearrangements can lead to the loss of tumour suppressors and/or the amplification of oncogenes and can contribute to cancer progression.

Gene fusions are a frequent result of chromosomal rearrangements and can result from translocations, deletions, inversions and duplications, as well as chromothripsis, a catastrophic genomic event leading to massive rearrangements of multiple chromosomes. Owing to the large number of gene fusions, the role in cancer cell migration and invasion could be the topic of another review, so we consider here some of the most common gene fusions. The first gene fusion to be discovered, BCR–ABL, is the result of a reciprocal translocation between chromosomes 22 and 9, and is detected in >96% of patients with chronic myeloid leukaemia. This fusion causes alterations in the actin cytoskeleton that promote the motility of chronic myeloid leukaemia cells, as demonstrated in various cell lines in vitro. The TMPRSS2–ERG gene fusion can arise from the inversion or interstitial deletion of chromosome 21q22 and is found in 50% of prostate cancers. This gene fusion leads to the overexpression of ERG (ETS-related gene), a transcription factor, which, in turn, promotes prostate tumour cell movement through Notch signalling or transcriptional activation of MMP9 and plexin A2, a semaphorin co-receptor. ERG overexpression as a result of the TMPRSS2–ERG gene fusion event has been demonstrated to promote EMT not only by activating TGF-β signalling but also by inducing WNT signalling. Other gene fusions also contribute to EMT. The MLL–AF9 translocation (t9;11) is found in acute myeloid leukaemia and promotes tumour invasion associated with the transcription factor ZEB1 in a long-term haematopoietic stem-cell-derived mouse model of acute myeloid leukaemia. Fusions between the oestrogen receptor gene (ESR1) and YAP1 (which encodes Yes1-associated transcriptional regulator) or PCDH11X (which encodes the cell adhesion protein protocadherin 11 X-linked) are associated with the induction of EMT and were shown to enhance the motility of T47D breast cancer cells in vitro and the metastasis of T47D xenografts.

Gene amplifications are frequently occurring events in many cancers and result in overexpression of genes—mainly oncogenes—that confer a growth or survival advantage on cancer cells. Indeed, ErbB2 gene amplification is one of the most frequent genetic events in breast cancer, resulting in the overexpression of HER2, which promotes cell proliferation predominantly through the activation of the mitogen-activated protein kinase (MAPK) pathway. However, ErbB2 gene amplification can also induce breast cancer cell migration and invasion through the HER2-mediated activation of the Rho GTPases Rac1 and Cdc42, master regulators of cytoskeletal dynamics. Overexpression of fibroblast growth factor receptor 1 (FGFR1) due to amplification of the corresponding gene FGFR1 promotes EMT and increases migration and invasion of H1581 NSCLC cells and DMS114 small-cell lung cancer cells in vitro by upregulating the expression of the transcription factor SOX2, one of the core operators of stemness and EMT. The amplification of wild-type EGFR and subsequent activation of the epidermal growth factor receptor (EGFR) contribute to the non-angiogenic invasive growth of glioblastoma in the patient-derived rat xenograft model probably through the induction of EMT and correlate with glioblastoma invasion in patients.

Amplification of growth factor receptor genes is not the only way to induce cancer cell invasion and migration. Amplification of chromosome region 11q13, which encompasses genes encoding regulators of the actin cytoskeleton and cell motility (e.g., cortactin, coflin and p21-activated kinase 1), occurs in 30–50% of head and neck squamous cell carcinomas (HNSCC). An in vitro study demonstrated that 11q13 amplification promotes the overexpression of cortactin, which binds to and activates the Arp2/3 actin-nucleating complex, leading to the increased migration and invasion of various HNSCC cell lines (UMSCC2, UMSCC19 and MSK921). By contrast, 11q13 amplification-driven overexpression of the PPFIA1 gene, which encodes liprin-a1, a protein potentially involved in cell–matrix interactions, suppresses migration and invasion of FaDu HNSCC cells in vitro. These results indicate the presence of both positive and negative regulators of cell motility in this chromosomal region. Amplification of another chromosome region, 11q22.1–q22.2, is often found in oral squamous cell carcinomas and is associated with lymph-node metastasis. This amplification leads to overexpression of the BirC3 gene, the protein product of which—cellular inhibitor of apoptosis (cIAP)2—enhances the migration and invasion of SCC29B oral squamous carcinoma cells in vitro. Additional studies have shown that amplification of chromosome regions harbouring non-coding RNAs also triggers tumour cell migration and invasion. Gene-amplification-driven long non-coding RNA (lncRNA) SNHG17 promotes the migration of AS49 and PC-9 NSCLC cells in vitro, whereas amplification of lncRNA PCAT6 is important for motility in HepG2 and SMMC-7721 hepatocellular carcinoma cells in vitro. Amplification and subsequent overexpression of miR-151 directly targets RhoGDIA, a putative metastasis suppressor, to promote the migration and invasion of Huh7 and SMMC-7721 hepatocellular carcinoma cells in vitro and the metastasis of SMMC-7721 cells. A member of the miRNA cluster in the chromosomal locus 7q31–34 that is frequently amplified in melanoma, stimulates the migration of SK-MEL-19 melanoma cells in vitro and increases the metastatic potential of B16F10 mouse melanoma cells.

GENE ALTERATIONS

In addition to harbouring chromosomal abnormalities, different cancers also contain an abundance of point mutations as well as
Gene alterations and cancer invasion. Various gene mutations can affect tumour cell migration and invasion. Genes responsible for genome maintenance are frequently mutated in cancers; however, only a few of them can influence tumour cell motility, the main player here being TP53 and its diverse mutant forms. Alterations in genes that play a role in cell survival affect a variety of cellular processes and signalling pathways underlying cell migration. Mutations in genes encoding regulators of the actin cytoskeleton, adhesion, proteolysis and EMT directly influence the ability of tumour cells to migrate and invade.

Gene insertions and deletions (indels). These gene alterations play a significant role in various stages of cancer metastasis, and invasion is no exception. Below, we outline those genes whose alteration affects the migration and invasion of tumour cells; they are divided into several groups, depending on their primary function (Fig. 3).

Genes involved in genome maintenance
Genes involved in maintaining genome stability are often mutated in cancer. Not only do loss-of-function (LOF) mutations of these tumour suppressors contribute to the acquisition of a mutator phenotype by tumour cells, but they can also affect cancer cell migration and invasion. Mutations in BRCA1 lead to dysregulation of the UbC9/caveolin-l/vascular endothelial growth factor (VEGF)/SIRTI/oestrogen receptor (ER)-α axis, promote EMT and trigger the migration of HCC1937 triple-negative breast cancer cells in vitro. The STAG2 gene, the protein product of which regulates centromere cohesion, is often mutated in various cancers. Most STAG2 mutations are truncating and, as shown in the U2OS osteosarcoma cell line, the loss of this gene leads to increased EMT-associated tumour cell migration in vitro, coincident with decreased expression of E-cadherin and increased expression of N-cadherin.

The best known ‘stabiliser’ of the genome and tumour suppressor, however, is p53. TP53 is often mutated in a wide variety of tumours, from carcinomas and sarcomas to lymphomas and leukaemias. Loss of p53 due to LOF mutations often leads to increased activity of the transcription factors Snail and Twist1, decreased expression of E-cadherin and induction of EMT. In addition, p53 loss activates Rho GTPases to increase cell migration, as shown in mouse embryonic fibroblasts and A375P melanoma cells in vitro. However, loss of TP53 might not always be sufficient to promote tumour cell invasion and metastasis, as shown in vivo in PVTT-1 hepatocellular carcinoma xenografts and transgenic mouse rhabdomyosarcoma model, indicating that gain-of-function (GOF) mutations of this gene are more potent activators of the metastatic cascade.

GOF mutations in TP53 cause an even more prominent effect on tumour cell invasiveness than do LOF mutations. Driver TP53 GOF mutations often occur at codons 175, 248 and 273 and endow the p53 protein with new abilities to regulate hundreds of different genes including other tumour suppressors. The mutants p53 R175H and R273H have been shown to bind to and inactivate the tumour suppressor p63 to form a mutant p53–p63 complex. This mutant complex suppresses Split and Hairy-related protein 1 (Sharp-1, a metastasis suppressor) and cyclin G2, and enhances TGF-β-mediated invasion and metastasis of MDA-MB-231 breast cancer cells in vitro and in vivo, as well as accelerates integrin recycling and activates signalling by the receptor tyrosine kinases EGFR and Met via Rab-coupling protein (RCP) in H1299 lung and MDA-MB-231 breast cancer cells. In these cancers, mutant p53 also promotes EGFR and Met signalling through the inactivation of a suppressor of invasion, Dicer ribonuclease, and enhances integrin and EGFR recycling and focal adhesion turnover by modulating components of the endosomal machinery. Inactivation of p63 by p53 mutants can also alter the expression of miRNAs involved in tumour cell migration. For example, mutant-p53-mediated upregulation of miR-155 leads to the increased migration and invasion of ZR-75-1 breast and H1299 lung cancer cells in vitro, and downregulation of tumour suppressor microRNA let-7i induced by the mutant p53–p63 complex leads to enhanced invasion of H1299 lung cancer cells in vitro. As demonstrated in H1299 lung cancer cells in vitro, formation of the mutant p53–p63 complex and the associated increase in cancer cell migration and invasion can be inhibited by the activating transcription factor 3 (ATF3) protein, which binds the mutant forms of p53 and thus facilitates p63 activation. It is important to note that the mutant p53–p63 complex and the mechanisms described above are not always required for the migration and invasion of tumour cells. Inactivation of Dicer ribonuclease mediated by mutant p53 can occur independently of the formation of the mutant p53–p63 complex.

In addition, GOF mutant forms of p53 can trigger EMT via overexpression of Twist, stabilisation of Slug and also by acting on ZEB1. Mutant p53 can enhance the expression of the A1AT protein, which promotes EMT-associated migration and invasion of H2009 lung cancer cells in vitro, and drives invasion of H2009 cells in the chick chorioallantoic membrane in vivo assay. The p53 R248Q mutant activates the phosphorylation of Stat3, which results in the enhanced EMT-dependent migration of HCT116 colorectal cancer cells and H1299 NSCLC cells in vitro. Mice with p53 mutations in addition to the loss of another tumour suppressor, RB1, develop mammary tumours with EMT features.

Numerous other studies have demonstrated the effect of GOF p53 mutations on a multitude of cell locomotion regulators. It should be noted, however, that p53 mutants can impact cell movement negatively as well as positively. For example, dominant-negative p53 mutants, such as R175H, R273H, R280K and R249S, can induce varying degrees of invasive potential in combination with the wild-type form of p53 in hTERT-HME1 (non-malignant) immortalised epithelial mammary cells. Thus, each of these p53 mutants may specifically affect the metastatic ability of cancer cells. In contrast, the p53 R248Q mutant negatively affects the migration of MDA-MB-231 breast and H1299 lung cancer cells in vitro and alters the distribution of MDA-MB-231 cells injected into zebrafish embryos, and contributes to mesenchymal–epithelial transition (the opposite of EMT). More research is therefore needed to understand the effects of different p53 GOF mutations on tumour cell motility and invasiveness.
Genes involved in cell survival

Similar to genome-maintenance regulators, driver genes that modulate cell proliferation and survival are frequently mutated in different cancers. These genes encode growth factor receptors and components of Ras and phosphatidylinositol 3-kinase (PI3K) signalling pathways.

A significant effect on cancer cell migration and invasion is exerted by alterations in the genes encoding various growth factor receptors. In addition to the amplification of genes encoding various growth factor receptors (described above), point mutations and indels in these genes can also affect the motility of tumour cells. The EGFR L858R mutation enhances the migration and invasion of A549, H1299 and CL1-0 lung cancer cells in vitro.95,96 Notably, however, HOG glioma cells with this mutation migrate slower in vitro than cells with wild-type EGFR. Probably, this is due to the fact that EGFR oncogene does not initially provide a selective advantage for HOG cells, while the EGFR mutation negatively affects cell growth and migration.90

Another mutant, EGFRvIII, is characterised by the loss of two extracellular domains owing to the deletion of exons 2–7, which renders the mutant receptor constitutively active and unable to bind ligands. EGFRvIII promotes the migration and invasion of glioblastoma cells through the induction of proteases, integrin signalling and other mechanisms.91–93 The so-called ‘gatekeeper’ V561M mutation in EGFRvIII confers resistance to EGFR inhibitors, as well as promoting the mesenchymal phenotype and enhancing the ability of H1581 NSCLC cells to migrate and invade in vitro.94 Activating mutations in FGF receptor 2 contribute to a loss of polarity and impaired directional cell migration, but promote invasion of HKE-293FT endometrial cancer cells in vivo.95

Mutations in Ras-family GTPases are very common in various cancers and significantly affect tumour progression.96 HRAS Q61R and NRAS Q61R driver mutations induce EMT and enhance the migration of Nthy-ori 3–1 thyroid cancer cells and MCF10A breast epithelial cells, respectively.97,98 Driver mutations in KRAS at position G12 promote EMT via Wnt/β-catenin and TGF-β signalling pathways in the iKAP mouse model of colorectal cancer in vivo99 and in various pancreatic cancer cell lines in vitro and in vivo.100,101 Moreover, the KRAS G12 and HRAS G12 mutants can modulate the function of the Rho GTPases RhoA, Rac1 and Cdc42 through the Ras and PI3K signalling pathways in the Caco-2 colorectal cancer cell line in vitro and thereby modulate migration and invasion.102 Overexpression of KRAS G12V leads to a decrease of collective invasion of MCF10A human mammary epithelial cells.103

Mutations in genes encoding downstream effectors of Ras GTPases also affect the ability of tumour cells to move. The BRAF V600E driver mutation occurs in almost half of all melanoma cases and enhances the kinase activity of the BRAF protein.104 The V600E mutation induces the migration and invasion of WM3211 melanoma cells in vitro and the migration of mouse melanoma in vivo by stimulating integrin signalling, actin protrusion formation and the expression of MMPs through activation of extracellular signal-regulated kinase (ERK)/MAPK.105 The BRAF V600E mutant also contributes to invasion of cancers other than melanoma. In thyroid cancer, the BRAF V600E mutant promotes cell movement through the nuclear factor (NF)-κB pathway as demonstrated in WRO and KTC-3 cell lines in vitro,106 or by mediating hypomethylation and subsequent overexpression of the gene encoding WAS/WASL-interacting protein family member 1 (WIPF1), as demonstrated in K1, OCUT1 and FTC133 cells in vitro and K1 cells in vivo.107 In the Caco-2 colorectal cancer cell line, BRAF V600E represses E-cadherin and enhances the activity of Rho GTPases.108 Other evidence also supports a role for BRAF mutations in EMT-associated tumour invasion.108,109

Mutations in the genes encoding ERK/MAPKs or MAPK/ERK kinases (MEKs) also modulate tumour cell movement. The ERK3 L290P/V mutation promotes the migration and invasion but not proliferation of H1299 and A549 NSCLC cells in vitro.110 Loss of MKK4 protein due to MAP2K4 LOF mutations enhances the invasion associated with peroxisome proliferator-activated receptor γ (PPARγ) of various lung cancer cell lines (344SQ, 393P and H2009) in vitro.111 PIK3CA and PTEN, which encode components of the PI3K signalling pathway, are among the most frequently mutated genes in various cancers.112 ES45K and H1047R mutations in the p110 catalytic subunit of PI3K, which confer constitutive activity, have been shown to promote the migration and invasion of colorectal,113 gastric,114 cervical115 and breast cancer116 and HNSCC cells.117 In NOK and EPC1 HNSCC cell lines, the expression of mutant PIK3CA together with the downregulation of p120 catenin induces tumour invasion in vitro, including in 3D organotypic cultures, through an increase in the expression of MMPs.118 PTEN LOF mutations are observed in various cancers119 and contribute to EMT and the dissemination of tumour cells.120 For example, deletion of PTEN leads to increased collective invasion of MCF10A cells in contrast to KRAS G12V overexpression as mentioned above. Interestingly, the double PTEN and KRAS mutant cells show decreased collective behaviour, suggesting that KRAS dominates the collective migration phenotype.121 GOF mutations in PTEN are also known to modulate tumour cell movement. For example, the A126G mutant promotes the migration of PC3 prostate cancer cells in vitro.122

Mutations in the genes encoding AKT and mammalian target of rapamycin (mTOR), which are involved in the PI3K signalling pathway, are rare in cancers.123 However, mutant forms of these proteins can still contribute to cancer cell migration and invasion. The AKT1 E17K mutation (0.6–2% frequency in NSCLC) enhances the migration and invasion of normal lung epithelial cells (BEAS-2B) by relocating the cyclin-dependent kinase inhibitor p27 into the nucleoplasm from the cytoplasm and inhibiting RhoA signalling.124 The same mutated form of AKT1 increases the migration and invasion of human mammary luminal (HMLER) but not myoepithelial (Bpler) cells.125 GOF mutations conferred by mutated mTOR occur with a frequency of no more than 1% for various types of cancer; some of these mutations (e.g., A1256G and G7076A) promote tumour cell migration and invasion in vitro.126 Mutations in other genes implicated in cell survival have also been reported to influence cell invasion. Retinoblastoma protein, encoded by RB1, is a well-known tumour suppressor that plays a role in controlling cell-cycle progression.127 Different mechanisms are involved in RB1 loss, including LOF mutations and deletions.128 The knockdown-mediated loss of RB1 expression in PC3, PC3-ML and LNCaP prostate cancer cells leads to the acquisition of an increased migratory and invasive capacity with decreased expression of E-cadherin in vitro.129 The loss of RB1 in MYC-overexpressing mouse mammary epithelial cells promotes invasion in vitro and enhances the invasive phenotype in MYC-overexpressing xenograft tumours.130 Moreover, RB1 suppression was demonstrated to stimulate collective invasion rather than single-cell invasion of basal-like breast carcinoma cells in vitro and in vivo. Importantly, Rb knockdown also induced expression of CD44, lymphovascular invasion, the release of circulating tumour cells and distant metastasis.131 The CAV1 gene encodes caveolin-1, a component of caveolae—specialised plasma membrane invaginations that regulate cell proliferation and migration.132 Using the highly metastatic Met-1 mammary epithelial cell line, it was demonstrated that the CAV1 P132L mutation, which occurs in 16% of breast cancers, promotes migration and invasion, and activates various signalling pathways involved in metastasis.133 The tyrosine phosphatase SHP2 (PTPN11) transmits signals from tyrosine kinase receptors and regulates cell proliferation. A GOF mutation in PTPN11 that confers a D61G substitution enhances the migration and invasion of MDA-MB-231 and MCF-7 breast cancer cells in vitro and the metastasis of both cell lines in vivo through the activation of the Ras and PI3K signalling pathways.134 Caspases are best known as essential mediators of the apoptotic programme...
and cell survival, but mutations in the CASP8 gene have been shown to accelerate migration and invasion of UM-SCC-47 HNSCC cells in vitro and their growth in vivo.\textsuperscript{134} Probably, it can be related to the catalytic and noncatalytic modes of action by which CASP8 influences cell adhesion and migration.\textsuperscript{135}

Actin cytoskeleton regulators

As mentioned above, Rho GTPases are key regulators of actin cytoskeleton remodelling. The best-studied Rho GTPases—Rac1 and RhoA—are often mutated in various types of cancer.\textsuperscript{136} \textit{RAC1} is the third most frequently mutated gene in melanoma after \textit{BRAF} and \textit{NRAS}.\textsuperscript{137} The \textit{RAC1} P29S driver mutation, which results from a \textit{C}→\textit{T} transition in response to UV damage, generates a more active form of Rac1. This mutant form is characterised by increased switching from the inactive, GDP-bound to the active, GTP-bound state, which enhances the interaction of Rac1 with its downstream effectors.\textsuperscript{138} The \textit{RAC1} P29S mutant promotes the migration of melanocytes\textsuperscript{139} and invasion of mouse embryonic fibroblasts in vitro.\textsuperscript{140} Although melanoma cells (104T cell line) with the \textit{RAC1} P29S mutation form lamellipodia more actively, this mutant negatively affects the formation of invadopodia and invadopodia-dependent matrix degradation in vitro. This can indicate that \textit{RAC1} P29S-harbouring melanoma cells have an enhanced migration, but attenuated invasion.\textsuperscript{141} \textit{RHOA} is a driver gene in many cancers, such as T-cell lymphoma and gastric cancer.\textsuperscript{142} LOF mutants of \textit{RHOA} (G17E, Y42C and Y42S) that are present in diffuse-type stomach cancers lead to the inactivation of RhoA–ROCK1 signalling and increased migration of MKN74 gastric tubular adenocarcinoma cells in vitro.\textsuperscript{143} Moreover, as shown in the orthotopic xenograft mouse model, MKN74 gastric cancer cells with \textit{RHOA} mutations are more invasive and acquire immune resistance.\textsuperscript{144}

Mutations of the genes encoding other Rho GTPases, such as Cdc42, Rac2, Rac3, RhoB and RhoC, are rare and their effect on tumour cell movement has not yet been characterised.\textsuperscript{142} However, as these Rho GTPases play an important role in the reorganisation of the actin cytoskeleton, their mutation probably also affects cancer cell migration.

The activity of Rho GTPases is positively regulated by Rho guanine nucleotide-exchange factors (GEFs) and negatively by Rho GTPase-activating proteins (GAPs);\textsuperscript{145} consequently, mutations in the genes encoding these Rho GTPase regulators significantly affect the migration and invasion of tumour cells. The \textit{PREX2} gene, which encodes a RhoGEF, is often mutated in metastatic solid tumours.\textsuperscript{146} The \textit{PREX2} S1113R mutant protein, present in patients with hepatocellular carcinoma, has been shown to promote the migration of Huh7 liver tumour cells in vitro.\textsuperscript{147} \textit{RGS7}, which encodes a Rho GTPase-activating protein, is a tumour suppressor that is mutated in melanoma. The \textit{RGS7} R44C mutation destabilises the protein, which thereby results in the enhanced motility of A375 melanoma cells in vitro.\textsuperscript{148} \textit{ARHGAP35}, which encodes a negative regulator of Rho GTPases, is mutated in 15% of endometrial tumours. \textit{ARHGAP35} GOF mutations (S866F and 866G→870) contribute to random MDA-MB-231 breast cancer cell migration in vitro, which might promote the exploratory behaviour of tumour cells.\textsuperscript{149} Rho GTPases regulate downstream signalling effectors such as ROCKs, p21-activated kinases (PAKs), the SCAR/WAVE complex, LIM kinase (LIMK), coflin and Arp2/3, which control actin cytoskeleton remodelling. Despite these effectors rarely being mutated in various cancers, it is logical to assume that mutations in their encoding genes, if they do occur, might affect the migration and invasion of tumour cells. Loss of the \textit{ABI1} gene (which encodes a component of the SCAR/WAVE complex) leads to the induction of EMT and increased migration and invasion of RWPE-1 benign prostate epithelial cells in 2D and 3D in vitro systems.\textsuperscript{150} However, these results contradict the general consensus that overexpression of the SCAR/WAVE complex is associated with increased cancer invasion and poor prognosis, as outlined by Molinie and Gautreau.\textsuperscript{151} The E329K mutant of PAK4 promotes the motility of PC3 prostate carcinoma cells in vitro,\textsuperscript{152} and GOF mutations in the \textit{ROCK1} gene promote mouse embryonic fibroblast migration in vitro.\textsuperscript{153} However, it is important to note that, as mentioned above, mutations in downstream effectors of Rho GTPases are rare in cancer, and the dysregulation of these effectors in tumour cells is predominantly caused by other mechanisms.\textsuperscript{154}

Genes involved in cell adhesion and ECM proteolysis

Changes in cell adhesion and proteolysis of the ECM are inextricably linked to cell movement.\textsuperscript{155} Again, the genes underlying these processes are rarely mutated in cancers; however, experimental data indicate the importance of their potential mutations in the movement of tumour cells.

Integrins play a big role in cell adhesion, and changes in their expression promote cancer invasion.\textsuperscript{155} Although integrins are frequently dysregulated in various types of cancer, integrin mutations are poorly studied, especially in terms of their effect on tumour cell migration.\textsuperscript{156} The integrin α1 mutant T188I, which is found in poorly differentiated human squamous cell carcinoma of the tongue, enhances cell spreading (anchoring to the substrate) and actin cytoskeleton assembly, but does not promote migration or invasion of mouse keratinocytes in vitro.\textsuperscript{157,158} Note that cell spreading and cell motility are mechanistically different phenomena despite outward similarities.\textsuperscript{159} Integrin α7 is frequently inactivated in prostate tumours and leiomyosarcoma due to truncating mutations in the corresponding gene, and expression of wild-type ITGA7 inhibits the migration of prostate cancer (PC3 and Du145) and SK-UT-1 leiomyosarcoma cells in vitro.\textsuperscript{160} Nevertheless, the effect of most integrin mutations on tumour cell migration and invasion remains unstudied.

Mutations in the genes encoding α-catenin (\textit{CTNNAL2} and \textit{CTNNAL3}) are characteristic of laryngeal squamous cell carcinoma and have been shown to promote tumour invasion of SCC-2 oral cancer cells in vitro.\textsuperscript{161} The adaptor protein paxillin (encoded by the \textit{PXN} gene), a key component of focal adhesions, was mutated in up to 9.4% of NSCLC cases analysed by Jagadeeswaran et al.\textsuperscript{162} The most frequent mutation, A127T, enhances focal adhesion and lamellipodia formation in HEK-293 human embryonic kidney cells in vitro,\textsuperscript{163} and promotes the invasion of HS22 NSCLC cells in vitro.\textsuperscript{164} EphB6 is a receptor for ephrin-B ligands that modulates cell adhesion and migration. The \textit{EphB6} Q926R mutation activates RhoA through the induction, via JNK signalling, of cadherin-11 expression, and increases the invasion of A549 lung, Huh7 liver and A375 skin cancer cells in vitro.\textsuperscript{165} The deletion of exon 33 in the gene encoding focal adhesion kinase (\textit{FAK}) confers a gain of function on the protein that enhances migration and invasion of MDA-MB-468 breast cancer cells in vitro.\textsuperscript{166} Onder et al. showed that truncating mutations in the \textit{CDH1} gene, that lead to the expression of a dominant-negative protein, promote cell migration and growth of HMLER cells in vitro and in vivo, but to a lesser extent than the shRNA-mediated loss of E-cadherin.\textsuperscript{167} Other studies showed that \textit{CDH1} mutations do not affect EMT or the motility of various breast cancer cell lines (MDA-MB-231, MCF-7, etc.) in vitro.\textsuperscript{168,169} All these data might indicate the cell-specific effect of \textit{CDH1} mutations.

Tumour cells must be able to degrade the ECM in order to penetrate the surrounding tissue and disseminate. It is therefore logical to assume that mutations in genes encoding proteases might alter the invasive potential of tumour cells. Similar to the situation regarding Rho GTPase effectors and integrins, most of the genes encoding various proteases, especially MMPs, are infrequently mutated in cancers; however, there are some data regarding the impact of their alterations on cancer cell migration and invasion. For example, mutations in the \textit{MMP8} gene, often found in melanoma, enhance the migration of immortalised
Table 1. Genetic alterations associated with migration and invasion of different cancer cells.

| Cancer                                | Genetic alterations                                      |
|---------------------------------------|---------------------------------------------------------|
| Breast cancer                         | Chromosomal instability: polyplody, ESR1–YAP1 and ESR1–PCDH11X fusions, ERBB2 amplification, LOH of 8p22 (DLC1) and LOH of 8p |
|                                       | Gene alterations: BRCAl, TP53, NRAS, PIK3CA, RB1, CAVI, PTPN11, ARHGPAP35, FAK, CDH1, ADAM12, ADPGK, PCGF6, PKP2, NUP93, SLC22A5 and TRIM21 |
| Colorectal cancer                     | Chromosomal instability: polyplody, trisomy of chromosomes 5, 7, 13 and 18 |
|                                       | Gene alterations: TP53, KRAS, BRAF, PIK3CA, APC and SMAD4 |
| Prostate cancer                       | Chromosomal instability: TMPRSS2–ERG fusion and LOH of 8p22 (DLC1) |
|                                       | Gene alterations: PTEN, RB1, AB11, PAK4 and ITGA7 |
| Non-small-cell lung cancer            | Chromosomal instability: FGFR1 and SNHG17 amplifications, and LOH of 8p22 (DLC1) and 1p32 (TGFB3) |
|                                       | Gene alterations: TP53, EGFR, FGFR1, ERK3, MAP2K4, AKT1, PKX, EPB16 and ND6 |
| Melanoma                              | Chromosomal instability: miR-182 amplification          |
|                                       | Gene alterations: TP53, BRAF, RAC1, RGS7, MMP8 and GRM3 |
| Head and neck squamous cell carcinoma | Chromosomal instability: 11q13 amplification            |
|                                       | Gene alterations: PIK3CA and CASP8                      |
| Oral squamous cell carcinoma          | Chromosomal instability: 11q22.1–q22.2 amplification    |
|                                       | Gene alterations: TGFBR2 and NOTCH1                     |

Transformed human Mel-STR melanocytes in vitro and in vivo. Surprisingly, wild-type MMP8 inhibits melanoma cell migration. Migration and invasion-suppressive role of MMP8 are also known in oral tongue squamous cell and breast carcinomas. Moreover, in breast cancer, MMP8 can prevent metastasis formation. The exact mechanisms of the suppressive effects of MMP8 are still unclear. Probably, MMP8 triggers migration- and invasion-suppressive molecular cascades through cleavage of various non-ECM substrates with specific regulatory functions. Similarly, mutations in the gene encoding a disintegrin-like and metalloproteinase domain with thrombospondin type 1 motifs (ADAMTS18) are potential drivers of melanoma and promote the migration of A375 melanoma cells in vitro and the metastasis of Mel-STR cells in vivo. Notably, however, evidence exists that mutations in protease genes can confer an inhibitory effect on the movement of tumour cells. Mutant forms of ADAMTS16 have been shown to inhibit the motility of A2780CP20 ovarian cancer cells in vitro and in vivo. Breast cancer-associated mutations in the ADAM12 gene interfere with the intracellular trafficking of the corresponding protein and inhibit the migration of mouse embryonic fibroblasts in vitro. In general, proteases (especially MMPs) are considered as potential druggable targets in anticancer therapy but whether their mutants can be therapeutically targeted is currently unknown, probably due to the fact that these genes are very rarely mutated in cancers. Furthermore, the enhanced migration of MMP8 mutant-immortalised melanocytes emphasises the need to assess the function of each MMP individually to define its precise role in cancer.

**EMT regulators**

As demonstrated above, mutant forms of many oncopgenes and tumour suppressors can modulate EMT through different mechanisms. But what about other regulators of EMT? Although mutations in genes encoding transcription factors that are involved in EMT (Twist, Snail, Slug and Zeb1) are known to be extremely rare in cancer, the activity of these transcription factors is regulated by other genes, mutations in which they can occur more frequently in various cancers. For example, mutations in the driver genes (ADPGK (encodes ADP-dependent glucokinase), PCGF6 (polycystin group RING finger protein 6), PKP2 (plakophilin 2), NUP93 (nucleoporin 93) and SLC22A5 (solute carrier family 22 member 5)) can affect EMT and promote MDA-MB-231 breast cancer cell migration in vitro. The gene encoding another EMT regulator, TRIM21, which promotes the proteasomal degradation of Snail and thereby suppresses migration and invasion, is rarely mutated in breast cancer (frequency <1%), but the R64Q mutation abrogates the ability of TRIM21 to mediate Snail degradation and thus promotes breast cancer cell invasion. GOF mutations in the TGF-β receptor II gene (TGFBR2) induce the re-localisation of E-cadherin from the cell membrane to the cytoplasm and overexpression of vimentin, and promote TGF-β signalling, migration and invasion of HSC-2 oral squamous cell carcinoma cells in vitro, contributing to aggressive cancer behaviour.

Mutations in the genes that encode Smad transcription factor proteins, which are key mediators of TGF-β signalling, can promote TGF-β-mediated EMT. Furthermore, driver mutations in the APC, CTNNB1 and NOTCH1 genes, and other components of the WNT and Notch signalling pathways, contribute to EMT in various cancers. Miscellaneous genes As a consequence of mutation, genes that are not directly related to the regulation of cell movement can sometimes acquire new functions and thus promote cancer cell migration and invasion. Missense and nonsense mutations in the mitochondrial gene ND6, which normally encodes a subunit of NADH dehydrogenase (ubiquinone), promote migration and invasion of A549 lung adenocarcinoma cells in vitro, probably via the increased generation of reactive oxygen species. Activating mutations in the GRM3 gene, which encodes a G-protein-coupled receptor, occur in melanoma and stimulate the migration of A375 melanoma cells in vitro, probably through phosphorylation of MEK.
movement hinges upon certain mutations or other, non-genetic triggers. Another important issue is the need to identify mutational drivers of invasion and metastasis, both universal and specific for different types of cancer. Analysis of the studies discussed in this review shows that some genes (TPS3, EGFR and PIK3CA) can be common for various cancers in terms of the effect of their mutations on tumour cell migration and invasion, whereas other genes are strongly specific for certain malignant tumours: for example, RAC1 and ADAMTS18 in melanoma, and APC in colorectal cancer (see Table 1). Even though some genes that are involved in cell motility are rarely mutated in cancers (such as downstream effectors of Rho GTPases and integrins), their mutations, no matter how infrequently they occur, might play a big role in driving cancer invasion. Moreover, each cancer is likely to be unique in its genetic landscape, and therefore mutational drivers important for invasion could vary significantly from tumour to tumour. Thus, further studies should be focused on the development of an atlas of mutational drivers of cancer invasion as an important step towards understanding the genetic subtleties that underlie tumour dissemination.

Approaches to analysing mutational drivers of cancer invasion

Different approaches can be used to identify and study mutational drivers of cancer invasion. Metastatic mouse models of various cancers are an effective way to identify genetic alterations that contribute to tumour cell migration, invasion and metastasis. A 2017 study used a metastatic model of colorectal cancer to demonstrate that pronounced migration of tumour cells depends on the combined effect of mutations in APC, KRAS, TP53 and SMAD4. It seems reasonable to analyse cancer genomes by focusing on the functionally significant mutations in genes that regulate critical processes in cell migration and invasion—for example, EMT, actin cytoskeleton remodelling, proteolysis and so on—and to validate their significance in vitro and in vivo. Another potential approach is to analyse the mutational landscape of tumour cells located in the invasive front, and to select for genetic alterations that are not present in the tumour core. For example, local invasion is a hallmark of malignant gliomas, making glioma cells a candidate model for finding drivers of cancer invasion. However, data also indicate that highly dynamic cells are present not only at tumour borders but also in the tumour core, as was demonstrated in NICD/p53– mouse intestinal cancer and orthotopic human glioblastoma model, which significantly reduces the chance of finding mutations that drive cancer invasion when comparing the invasive front with the tumour core. In this case, it therefore seems reasonable to compare the mutational landscape of invasive and non-invasive tumour cells within the same tumour. Specific molecular markers could potentially be used to distinguish motile tumour cells from non-motile tumour cells in the primary tumour, and meticulous examination of the genomic landscape of such cells could uncover new mutational drivers of cancer invasion. However, no effective and reliable markers to help identify truly motile tumour cells currently exist.

In our studies, we have shown that the intratumoural morphological heterogeneity of invasive ductal carcinoma of the breast (now classified as invasive carcinoma of no special type) is a reflection of various patterns of tumour cell invasion. In particular, breast cancer cells can exist as single entities or be arranged in either small groups (2–5 cells) or multicellular structures: tubular, alveolar, solid, trabecular and torpedo-like structures (Fig. 4). Tubular and alveolar structures are transcriptionally similar and demonstrate a similar expression of epithelial and mesenchymal markers. Solid structures show an increase in mesenchymal traits but retain epithelial features. Trabecular structures, small groups of tumour cells and single tumour cells all display a pronounced mesenchymal phenotype and a dramatic decrease in epithelial traits, as well as significant enrichment of cancer invasion signalling pathways. The presence of alveolar and trabecular structures in breast tumours is associated with increased lymph-node metastasis and distant recurrence in patients treated with neoadjuvant chemotherapy. Distant metastases are also frequently detected in breast cancers with single tumour cells with epithelial-like morphology, and in breast cancers that express kinesin-14 (KIF14) and mitochondria-eating protein (Mieap), but lack ezrin (EZR) at the tips of torpedo-like structures. The nature of
torpedo-like structures, e.g., their EMT features, remains to be elucidated; however, KIF14, Mieap and EZR proteins are known to be important regulators of tumour cell migration and invasion. Based on all these results, we assumed that tubular and alveolar structures show decreased invasive potential, whereas solid, trabecular and torpedo-like structures, as well as small groups of tumour cells and single tumour cells, are highly invasive. The intratumoural morphological heterogeneity of breast cancer is therefore an attractive model for detecting mutational drivers of tumour cell invasion—for example, by comparing the genomic landscapes of highly invasive and less invasive morphological structures. Moreover, comparative analysis of multicellular structures (e.g., solid, trabecular or torpedo-like structures) against single tumour cells might provide information regarding genetic mutations that are involved in collective and individual modes of cancer invasion.

CONCLUSIONS AND DISCUSSION

Different chromosomal and gene aberrations influence cancer cell migration and invasion. CIN affects cancer cell movement through mechanisms associated with polyploidy and aneuploidy, as well as with gene fusion and amplification. Gene alterations trigger or suppress the spread of cancer cells in several ways, by influencing genes that affect genome maintenance, cell survival, actin cytoskeleton remodelling, EMT, adhesion and proteolysis. Such genetic drivers are of particular interest as potential prognostic markers and targets for anti-metastatic therapy. Indeed, some of the mutational drivers discussed in this review have already been established as potential targets for anticancer therapy—p53 hotspot mutations, EGFR mutations and PI3K p110α E545K and H1047R mutants. The main objective of anticancer therapy is to stop tumour growth and to kill cancer cells. However, another therapeutic approach, which is receiving ever-increasing interest, is to block the ability of tumour cells to invade and metastasise. Migrastics are a novel class of anticancer drugs aimed at attenuating cancer cell migration by targeting the signalling pathways and downstream effectors that are involved in cell motility. The downside of these therapeutics is that they can be toxic for all types of moving cells—for example, fibroblasts, keratinocytes and leukocytes. In this regard, mutational drivers of cancer invasion could constitute especially interesting targets for migrastics as these genetic alterations are present only in tumour cells. Nevertheless, this issue requires a great deal of further research.

Further studies are also needed to explore known genetic mutations as well as to identify novel ones that affect invasion in various cancers, and to understand the number, combination and sequence of potential driver mutations that are required to promote tumour cell movement. Moreover, it must be demonstrated whether such mutational drivers are capable of promoting the motility of tumour cells independently of other prometastatic factors, such as the tumour microenvironment, epigenetic alterations and gene expression changes, or if genetic alterations serve merely as a build-up for other determinants of cancer invasion and metastasis. One way or another, it is simply not enough to study the problem of cancer invasion and metastasis from one narrow point of view. An integrated approach, which combines the careful and considered examination of tumour cell motility at the genome, epigenome, transcriptome and proteome levels, is needed for a comprehensive understanding of cancer invasion and metastasis.

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18. Durkin, M. E., Yuan, B.-Z., Zhou, X., Zimonjic, D. B., Lowy, D. R., Thorgeirsson, S. S. et al. DLC-1: a Rho GTPase-activating protein and tumour suppressor. J. Cell. Mol. Med. 11, 1185–1207 (2007).

19. Cai, Y., Crowther, J., Pastor, T., Abbasi Asbagh, L., Baetti, M. F., De Troyer, M. et al. Loss of chromosome 8p governs tumor progression and drug response by altering lipid metabolism. Cancer Cell 29, 751–766 (2016).

20. Fingar, D. M., Turlay, R. S., Dong, M., How, T., Fields, T. A. & Blobe, G. C. TJPIII suppresses non-small cell lung cancer invasiveness and tumorigenicity. Carcinogenesis 29, 528–535 (2008).

21. Davoli, T. & de Lange, T. The causes and consequences of polyplody in normal development and cancer. Annu. Rev. Cell Dev. Biol. 27, 585–610 (2011).

22. Ganem, N. J., Storchova, Z. & Pellman, D. Tetraploidy, aneuploidy and cancer. Curr. Opin. Genet. Dev. 20, 157–2991 (2010).

23. Niu, N., Zhang, J., Zhang, N., Mercado-Uribe, I., Tao, F., Han, Z. et al. Linking genomic reorganization to tumor initiation via the giant cell cycle. Oncogene e5 (2018).

24. Mirzayans, R., Andrais, B. & Murray, D. Roles of polyploid/multinucleated giant cancer cells in metastasis and disease relapse following anticancer treatment. Cancers 10, 1–11 (2018).

25. Zhang, S., Zhang, D., Yang, Z. & Zhang, X. Tumor budding, micropapillary pattern, and polyplody giant cancer cells in colorectal cancer: current status and future prospects. Stem Cells Int. 2016, 1–8 (2016).

26. Fei, F., Zhang, D., Yang, Z., Wang, S., Wang, X., Wu, Z. et al. The number of polyplody giant cancer cells and epithelial-mesenchymal transition-related proteins are associated with invasion and metastasis in breast human cancer. J. Exp. Clin. Cancer Res. 34, 1–13 (2015).

27. Fei, F., Zhang, M., Li, B., Zhao, L., Wang, H., Liu, L. et al. Formation of polyplody giant cancer cells involves in the prognostic value of neoadjuvant chemoradication in locally advanced rectal cancer. J. Oncol. 2019, 1–15 (2019).

28. Niu, N., Mercado-Uribe, I. & Liu, J. Dedifferentiation into blastomere-like cancer stem cells via formation of polyplody giant cancer cells. Oncogene 36, 4887–4890 (2017).

29. Xuan, B., Gohsh, D., Chen, E. M., Clifton, E. M. & Dawson, M. R. Dysregulation in actin cytoskeletal organization drives increased stiffness and migratory persistence in polyplody giant cancer cells. Sci. Rep. 8, 1–13 (2018).

30. Fei, F., Liu, K., Li, C., Du, J., Wei, Z., Li, B. et al. Molecular mechanisms by which S100A4 regulates the migration and invasion of PGCCs with their daughter cells. Oncotarget 25115, 25130 (2017).

31. Wangsa-D, Quintanilla, I., Torabi, K., Vila, F. et al. Molecular mechanisms by which S100A4 regulates the migration and invasion of PGCCs with their daughter cells. Oncotarget 5, 390–400 (2016).

32. Vasudevan, A., Baruah, P. S., Smith, J. C., Wang, Z., Sayles, N. M., Andrews, P. et al. Gene amplification-driven long noncoding RNA SNNHG1 regulates cell proliferation and migration in human non-small-cell lung cancer. Mol. Ther. Nucleic Acids 7, 405–413 (2019).

33. Chen, S., Ren, Y., Qian, Q., Wang, X., Chang, Y., Ju, S. et al. Gene amplification-driven cancer-a tests long noncoding RNA SNNHG1 regulates cell proliferation and migration in human non-small-cell lung cancer. Mol. Ther. Nucleic Acids 7, 405–413 (2019).

34. Ding, J., Huang, S., Wu, S., Zhao, Y., Liang, L., Yan, M. et al. Gain of mir-151 on chromosome 8q24.3 facilitates tumour cell migration and spreading through downregulating RhoGDI. Nat. Cell Biol. 12, 390–399 (2010).

35. Segura, M. F., Hanniford, D., Menendez, S., Reavie, L., Zou, X., Alvarez-Diaz, S. et al. Aberrant miR-182 expression promotes melanoma metastasis by repression of FOXO3 and microphthalmia-associated transcription factor. Proc. Natl Acad. Sci. USA 106, 1814–1819 (2009).

36. Nguyen, D. X. & Massagué, J. Genetic determinants of cancer metastasis. Nat. Rev. Genet. 8, 341–352 (2007).

37. Xu, J., Shumate, C., Qin, Y., Reddy, V., Burnam, Y., Lopez, V. et al. A novel BcR9-dependent pathway regulates SIRT1—ERα Axis and BRCA1-associated TNBC metastasis. Integr. Mol. Med. 4, 139–148 (2017).

38. Xu, J., Foothman, A., Qin, Y., Aysola, K., Black, S., Reddy, V. et al. BRCA1 mutation leads to deregulated BcR9 levels which triggers proliferation and migration of patient-derived high grade serous ovarian cancer and triple negative breast cancer cells. Int. J. Chronic Dis. 2, 31–38 (2016).

39. Nie, Z., Gao, W., Zhang, Y., Hou, Y., Liu, J., Li, Z. et al. STAG2 loss-of-function mutation induces PD-L1 expression in U2OS cells. Ann. Transl. Med. 7, 127–127 (2019).

40. Oliver, M., Hollstein, M. & Hainaut, P. TP53 mutations in human cancers: origins, consequences, and clinical use. Cold Spring Harb. Perspect. Biol. 2, 1–17 (2010).

41. Chang, C.-J., Chao, C.-H., Xie, W., Yang, J.-Y., Xiong, Y., Li, C.-W. et al. p53 regulates epithelial-mesenchymal transition and stem cell properties through modulating miRNAs. Nat. Cell Biol. 13, 317–322 (2011).

42. Yang-Hartwich, H., Teda, R., Roberts, C. M., Goodner-Bingham, J., Cardenas, C., Gurea, M. et al. p53–Phir2 Complex Promotes Twist1 Degradation and Inhibits EMT. Mol. Cancer Res. 17, 153–164 (2019).

43. Wang, Z., Jiang, Y., Guan, D., Li, J., Yin, H., Pan, Y. et al. Critical roles of p53 in epithelial-mesenchymal transition and metastasis of hepatocellular carcinoma cells. PloS ONE 8, e72846 (2013).
Different mutant/wild-type p53 combinations cause a spectrum of increased cancer type-specific migration and invasiveness.

Mutant p53 upregulates alpha-1 antitrypsin expression and promotes invasion in glioblastoma: combination therapies for an effective treatment. Int. J. Mol. Sci. 18, 1–19 (2017).

Sangar, V., Funk, C. C., Kusebauch, U., Campbell, D. S., Moritz, R. L. & Price, N. D. Quantitative proteomic analysis reveals effects of epidermal growth factor receptor (EGFR) on invasion-promoting proteins secreted by glioblastoma cells. Mol. Cell. Proteom. 13, 2618–2631 (2014).

Liu, M., Yang, C., Sun, L., Mei, C., Yao, W. et al. The effect of epidermal growth factor receptor variant III on glioma cell migration by stimulating ERK phosphorylation through the focal adhesion kinase signaling pathway. Arch. Biochem. Biophys. 502, 89–95 (2010).

Ryan, M. R., Sohl, C. D., Luo, B. & Anderson, K. S. The FGFR1 V561D gatekeeper mutation drives AZD4547 resistance through STAT3 activation and EMT. Mol. Cancer Res. 17, 532–543 (2019).

Steibens, S. J., Li, R. J., Adams, M. N., Perry, S. R., Haass, N. K., Bryant, D. M. et al. FGFR2-inactivating mutations disrupt cell polarity to potentiate migration and invasion in endometrial cancer cell models. J. Cell Sci. 131, 1–16 (2018).

Hobbs, G. A., Der, C. J. & Rossman, K. L. RAS isoforms and mutations in cancer at a glance. J. Cell Sci. 129, 1287–1292 (2016).

Demir, D. E., Afanaseyeva, M. A., Uvarova, A. N., Prokofjeva, M. M., Gorbachova, A. M., Ustianova, A. S. et al. Constitutive expression of NRAS with Q61R driver mutation activates processes of epithelial–mesenchymal transition and leads to substantial transcriptome change of Nthy-ori 3–1 thyroid epithelial cells. Biochem. (Mosc.) 84, 416–425 (2019).

Geyer, F. C., Li, A., Papasiantasiou, A. D., Smith, A., Selenica, P., Burke, K. A. et al. Recurrent hotspot mutations in HRAS Q61 and PI3K-AKT pathway genes as drivers of breast adenomyoepithelomas, Nat. Commun. 9, 1–16 (2018).

Boutin, A. T., Liao, W., Wang, M., Hwang, S. S., Karpinets, T. V., Cheung, H. et al. Oncogenic Kras drives invasion and maintains metastases in colorectal cancer. Genes Dev. 31, 370–382 (2017).

Padavano, J., Henkhuis, R. S., Chen, H., Skovar, B. A., Cui, H. & Ignatenko, N. A. Mutant K-RAS promotes invasion and metastasis in pancreatic cancer through GFAP signaling pathways. Cancer Metastasis Rev. 8, 95–113 (2015).

Xu, W., Wang, Z., Zhang, W., Qian, J., Li, H., Kong, D. et al. Mutated K-ras activates Map2k4 functions as a tumor suppressor in lung adenocarcinoma and inhibits tumor cell invasion by decreasing peroxisome proliferator-activated receptor 2 expression. Mol. Cell. Biol. 31, 4270–4285 (2011).

Samuels, Y. & Waldman, T. Oncogenic mutations of PIK3CA in human cancers. Curr. Top. Microbiol. Immunol. 347, 21–41 (2010).
113. Samuels, Y., Diaz, L. A., Schmidt-Kittler, O., Cummins, J. M., DeLong, L., Cheong, I. et al. Mutant PIK3CA promotes cancer growth and invasion of human cancer cells. Cancer Cell 7, 561–573 (2005).

114. Kim, J. W., Lee, H. S., Nam, K. H., Ahn, S., Kim, J. W., Ahn, S. H. et al. PIK3CA mutations are associated with increased tumor aggressiveness and Akt activation in gastric cancer. Oncotarget 8, 90948–90958 (2017).

115. Aouad, W., Merry, C. D., Wang, C., Saba, E., McIntyre, J. B., Fang, S. et al. Phosphatidylinositol-3-kinase (PIK3CA) E545K mutation confers cisplatin resistance and a migratory phenotype in cervical cancer cells. Oncotarget 7, 82424–82439 (2016).

116. Fang, H., Flinn, R., Patsialou, A., Wyckoff, J., Rousou, E. T. T., Wu, H. et al. Differential enhancement of breast cancer cell motility and metastasis by helical and kinase domain mutations of class IA phosphoinositide 3-kinase. Cancer Res. 69, 8868–8876 (2009).

117. Murugan, A. K., Thong, N., Fukui, Y., Munirajan, A. K. & Tsuichida, N. Oncogenic mutations of the PIK3CA gene in head and neck squamous cell carcinomas. Int. J. Oncol. 32, 101–111 (2008).

118. Kidacki, M., Lehman, H. L., Green, M. V., Warrick, J. J. & Stairs, D. B. p120-catemin downregulation and PIK3CA mutations cooperate to induce invasion through MMP1 in HNSCC. Mol. Cancer Res. 15, 1398–1409 (2017).

119. Álvarez-García, V., Tawil, Y., Wise, H. M. & Leslie, N. R. Mechanisms of PTEN loss in cancer: It’s all about diversity. Semin. Cancer Biol. 39, 66–79 (2019).

120. Perumal, E., So Youn, K., Sun, S., Seung-Hyun, J., Suji, M., Jieying, L. et al. PTEN mutations of the PIK3CA gene in head and neck squamous cell carcinomas. J. Oncol. 82439 (2016).

121. Álvarez-García, V., Tawil, Y., Wise, H. M. & Leslie, N. R. Mechanisms of PTEN loss in cancer: It’s all about diversity. Semin. Cancer Biol. 39, 66–79 (2019).

122. Costa, H. A., Leitner, M. G., Soo, M. L., Marvantonio, A., Rychkova, A., Johnson, J. R. et al. Discovery and functional characterization of a neurotrophic PTEN mutation. Proc. Natl. Acad. Sci. USA 112, 13976–13981 (2015).

123. Mundi, P. S., Sachdev, J., McCourt, C. & Kinlsey, K. AKT in cancer: new molecular insights and advances in drug development. Br. J. Clin. Pharmacol. 80, 943–956 (2016).

124. Marco, C. De, Malanga, R., Rinaldo, N., Vita, F., De, Scrima, M., Lovisa, S. et al. Mutant AKT1-E17K is oncogenic in lung epithelial cells. Oncotarget 6, 39634–39650 (2015).

125. Salhia, B., Cott, C., Van, Tesoriere, G. & Vento, R. RB1 in cancer: different functions and characterization of two novel oncogenic mTOR mutations. Oncogene 38, 5211–5226 (2019).

126. Davis, M. J., Ha, B. H., Holman, E. C., Halaban, R., Schlessinger, J. & Boggon, T. J. RAC1P295S is a spontaneously activating cancer-associated GTPase. Proc. Natl. Acad. Sci. USA 110, 912–917 (2013).

127. Knudsen, E. S., McClendon, A. K., Franco, J., Ertel, A., Fortina, P. & Witkiewicz, A. K. Rho GTPases in cancer: friend or foe? Anneo, A., Tesoriere, G. & Vento, R. RB1 in cancer: different functions and characterization of two novel oncogenic mTOR mutations. Oncogene 38, 5211–5226 (2019).

128. Kruthammer, M., Yoneyama, H., Ahn, B. H., Evans, P., Barili, A., Mischler, J. P. et al. Exome sequencing identifies recurrent somatic RAC1 mutations in melanoma. Nat. Genet. 44, 1006–1014 (2012).

129. Murugan, A. K., Liu, R. & Xing, M. Identification and characterization of two novel oncogenic mTOR mutations. Oncogene 38, 5211–5226 (2019).

130. Kim, K.-J., Godarova, A., Seedle, K., Kim, M.-H., Ince, T. A., Wells, S. I. et al. Rb domain mutations of class IA phosphoinositide 3-kinase. Cancer Res. 70, 3018–3027 (2010).

131. Grande-García, A., Echarri, A., de Rooij, J., Alderson, N. B., Waterman-Storer, C. M., Cooper, J. & Giancotti, F. G. Integrin signaling in cancer: mechanotransduction, stromness, epithelial plasticity, and therapeutic resistance. Cancer Cell 35, 71–87 (2019).

132. McTavish, T., Taji, H., Tatsuguchi, T., Shirai, T., Osaki, K., Matsuura, S. et al. DOCK1 inhibition suppresses cancer cell invasion and macrophagocytosis induced by self-activating Rac1P295S mutation. Biochem. Biophys. Res. Commun. 497, 298–304 (2018).

133. Revach, O. Y., Winograd-Katz, S. E., Samuels, Y. & Geiger, B. The involvement of mutant RAC1 in the formation of invadopodia in cultured melanoma cells. Exp. Cell Res. 343, 82–88 (2016).

134. Porter, A. P., Papaioannou, A. & Malliri, A. Deregulation of Rho GTPases in cancer. Small GTPases 7, 123–138 (2016).

135. Nishizawa, T., Nakano, K., Harada, A., Kakikuchi, M., Funahashi, S. I., Suzuki, M. et al. DGC-specific RHOA mutations maintained cancer cell survival and promoted cell migration via ROCK inactivation. Oncotarget 9, 23198–23207 (2018).

136. Moon, S. Y. & Zheng, Y. Rho GTPase-activating proteins in cell regulation. Trends Cell Biol. 13, 12–23 (2003).

137. Robinson, D. R., Wu, Y., Lonigro, R. J., Vats, P., Cobain, E., Everett, J. et al. Integrative clinical genomics of metastatic breast cancer. Nature 548, 297–303 (2017).

138. Davis, M. J., Ha, B. H., Holman, E. C., Halaban, R., Schlessinger, J. & Boggon, T. J. RAC1P295S is a spontaneously activating cancer-associated GTPase. Proc. Natl. Acad. Sci. USA 110, 912–917 (2013).

139. Knudsen, E. S., McClendon, A. K., Franco, J., Ertel, A., Fortina, P. & Witkiewicz, A. K. Rho GTPases in cancer: friend or foe? Anneo, A., Tesoriere, G. & Vento, R. RB1 in cancer: different functions and characterization of two novel oncogenic mTOR mutations. Oncogene 38, 5211–5226 (2019).

140. McGrath, J. L. Cell spreading: the power to simplify. Curr. Biol. 17, 868–694 (2007).

141. Bonuccelli, G., Casimiro, M. C., Sotgia, F., Wang, C., Li, C., Katiyar, S. et al. Caveolin-1 (P132L), a common breast cancer mutation, confers mammary cell migration through Src kinase and Rho GTPases. J. Cell. Physiol. 228, 683–694 (2013).

142. Kim, K.-J., Godarova, A., Seedle, K., Kim, M.-H., Ince, T. A., Wells, S. I. et al. Rb suppresses collective invasion, circulation and metastasis of breast cancer cells in CD44-dependent manner. PLoS ONE 8, e80590 (2013).

143. Grande-Garcia, A., Echarri, A., de Rooij, J., Alderson, N. B., Waterman-Storer, C. M., Valdivielso, J. M. et al. Caveolin-1 regulates cell polarization and directional migration through Src kinase and Rho GTPases. J. Cell. Biol. 177, 683–694 (2007).

144. Bonuccelli, G., Casimiro, M. C., Sotgia, F., Wang, C., Li, C., Katiyar, S. et al. Caveolin-1 (P132L), a common breast cancer mutation, confers mammary cell invasiveness and defines a novel stem cell/mетastasis-associated gene signature. Am. J. Pathol. 174, 1650–1662 (2009).

145. Fu, Z., Wang, X., Fang, H., Liu, Y., Chen, D., Zhang, Q. et al. A tyrosine phosphatase SHP2 gain-of-function mutation enhances malignancy of breast carcinoma. Oncotarget 7, 5664–5676 (2016).

146. Keller, N., Ozmadenci, D., Ichim, G. & Stupack, D. Caspase-8 function, and phosphorylation, in cell migration. Semin. Cell Dev. Biol. 80, 105–117 (2018).

147. Svensmark, J. H. & Brakebusch, C. Rho GTPases in cancer: friend or foe? Oncogene 38, 7447–7456 (2019).

148. De, P., Aske, J. C. & Dey, N. RAC1 takes the lead in solid tumors. Cells 8, 382 (2019).
165. Fang, X. Q., Liu, X. F., Yao, L., Chen, C. Q., Gu, Z. D., Ni, P. H. et al. Somatic mutational analysis of FAK in breast cancer: a novel gain-of-function mutation due to deletion of exon 33. Biochem. Biophys. Res. Commun. 443, 363–369 (2016).

166. Onder, T. T., Gupta, P. B., Mani, S. A., Yang, J., Lander, E. S. & Weinberg, R. A. Loss of E-cadherin promotes metastasis via multiple downstream transcriptional pathways. Cancer Res. 68, 3645–3654 (2008).

167. Van Horsen, R., Holletelle, A., Rens, J. A. P. R., Eggermont, A. M. M., Schuttte, M. & Hagen, T. L. M. E-cadherin promoter methylation and mutation are inversely related to motility capacity of breast cancer cells. Breast Cancer Res. Treat. 136, 365–372 (2012).

168. Lombaerts, M., van Wezel, T., Philippo, K., Dierssen, J. W. F., Zimmerman, R. M. E., Juurikka, K., Butler, G. S., Salo, T., Nyberg, P. & Åström, P. The role of MMP8 in breast cancer metastasis. Breast Cancer Res. Treat. 136, 1047–1058 (2012).

169. Gutiérrez-Fernández, A., Fueyo, A., Folgueras, A. R., Garabaya, C., Pennington, C. J., Dyczynska, E., Syta, E., Sun, D. & Zolkiewska, A. Breast cancer-associated mutations in ADAMTS16 mutations sensitize ovarian cancer cells to platinumbased chemotherapy. Oncotarget 8, 88410–88420 (2017).

170. Dyczynska, E., Syta, E., Sun, D. & Zolkiewska, A. Breast cancer-associated mutations in metalloproteinase disintegrin ADAM12 interfere with the intracellular trafficking and processing of the protein. Int. J. Cancer 122, 2634–2640 (2008).

171. Etemadi, A., Ayelalbegian, H. T., Negahdari, B., Mazloomi, M. A., Daraei, H., Daraei, N. et al. Role of protease and protease inhibitors in cancer progression and pathogenesis. Biomed. Pharmacother. 86, 221–231 (2017).

172. Winer, A., Adams, S. & Mignatti, P. Matrix metalloproteinase inhibitors in cancer therapy: turning past failures into future successes. Mol. Cancer Ther. 16, 1147–1155 (2018).

173. Zhao, M., Kong, L., Liu, Y. & Qiu, H. DkBMET: an epithelial-mesenchymal transition associated gene resource. Sci. Rep. 5, 1–14 (2015).

174. Lee, J.-H., Zhao, X.-M., Yoon, I., Lee, J. Y., Kwon, N. H., Wang, Y.-Y. et al. Integrative analysis of mutational and transcriptional profiles reveals driver mutations of metastatic breast cancers. Cell Discov. 2, 16025 (2016).

175. Jin, Y., Zhao, X., Zhang, Q., Zhang, Y., Fu, X., Hu, X. et al. Cancer-associated mutation abolishes the impact of TRIM21 on the invasion of breast cancer cells. J. Cell Biol. 192, 782–798 (2010).

176. Park, I., Son, H. K., Che, Z. M. & Kim, J. A novel gain-of-function mutation of TGF-β receptor II promotes cancer progression via delayed receptor internalization in oral squamous cell carcinoma. Cancer Lett. 315, 161–169 (2012).

177. Park, S., Yang, M.-K., Park, Y., Hong, E., Hong, C. P., Park, J. et al. Identification of epithelial-mesenchymal transition-related target genes induced by the mutation of Smad3 linker phosphoetylation. J. Cancer Prev. 23, 1–10 (2018).

178. Wang, Z., Li, Y., Zhan, S., Zhang, L., Zhang, S., Tang, Q. et al. SMAD4 Y353C promotes the progression of PDAC. BMC Cancer 19, 1–12 (2019).

179. Gao, C., Wang, Y., Broadrus, D., Sun, L., Xue, F. & Zhang, W. Exon 3 mutations of CTNNB1 drive tumorigenesis: a review. Oncotarget 9, 5492–5508 (2018).

180. Kawasaki, Y., Sato, R. & Akiyama, T. Mutated APC and Asef are involved in the aggressiveness of oral tongue squamous cell carcinoma. Br. J. Cancer 94, 661–671 (2006).

181. Alam, S. H., Al-Majed, S. A., Al-Ammar, S. A., Al-Turki, H. A., El Shafei, A. et al. Mutational analysis of mutational and transcriptional pro...