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1 Mutational drivers of cancer cell migration and invasion

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3 Running title: Mutations and cancer invasion

4

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17

18 **Abstract**

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

31

32 Key words: cancer, migration, invasion, mutation

33

34 **BACKGROUND**

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

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

56 The role of genetic alterations in tumour cell migration and invasion has received
57 undeservedly little attention comparing to epigenetic and transcriptional mechanisms of cell
58 motility. Despite the huge amount of experimental data regarding the effect of genetic mutations
59 on cancer invasion, only a few reviews exist, most of which focus mainly on the tumour

60 suppressor p53.^{6,7} In this review, we summarise published data outlining chromosomal instability
61 (CIN) and gene alterations that impinge on some of the molecular components that are crucial
62 for cancer cell migration and invasion. We also discuss the main difficulties encountered in
63 identifying genetic alterations that drive cancer invasion and suggest potential models and
64 approaches to overcome these problems. Finally, we underscore the significance of identifying
65 mutational drivers of cancer invasion as potential therapeutic targets for the prevention of
66 metastatic disease.

67

68 **Chromosomal instability**

69 CIN, which includes changes in the number of chromosomes as well as their rearrangement, is
70 observed in many tumour types and is associated with tumour progression, as described in Box
71 1.⁸ For example, as shown in MDA-MB-231 triple-negative breast cancer cells *in vitro* and *in*
72 *vivo*, CIN can induce the transcriptional transition of tumour cells to a mesenchymal state
73 characterised by increased migratory and invasive behaviour with the activation of inflammatory
74 pathways.⁹ By increasing inflammation, CIN can also promote cancer metastasis.^{9,10} It is worth
75 noting, however, that CIN can influence the invasive and metastatic potential differently,
76 depending on the molecular landscape of tumour cells and their microenvironment (reviewed in
77 ¹⁰).

78 Two types of CIN can be distinguished (Fig. 2): numerical CIN, which is determined by the gain
79 or loss of whole chromosomes (aneuploidy) and chromosome sets (polyploidy); and structural
80 CIN, which involves fractions of chromosomes and can result in gene fusions, amplifications and
81 other alterations.⁸ In both cases, loss of heterozygosity (LOH) — defined as the loss of one allele
82 caused by deletion, mitotic recombination, gene conversion or loss of a chromosome — can
83 arise.¹¹ LOH is a common alteration in cancer; it results in haploinsufficiency or loss of gene
84 expression, and frequently affects tumour suppressor genes, thereby contributing to
85 tumourigenesis. In addition, LOH — alone, or together with other genetic or epigenetic

86 alterations — can influence the ability of cancer cells to invade.^{12,13} For example, LOH of the
87 8p22 chromosomal region (*DLCL1*, which encodes a Rho GTPase-activating protein) promotes
88 migration and invasion of breast,¹⁴ lung,¹⁵ prostate¹⁶ and liver¹⁷ cancer cells *in vitro*.¹⁸ LOH of
89 the 8p region leads to changes in lipid metabolism, which, in turn, increases the motility and
90 invasiveness of MCF10A breast cells *in vitro*.¹⁹ Loss of the expression of *TGFBR3*, which
91 encodes TGF- β R3, due to LOH of the 1p32 region enhances migration and invasion of A549
92 non-small cell lung cancer (NSCLC) cells *in vitro*.²⁰

93

94 **Numerical CIN.** Gain or loss of whole chromosomes (aneuploidy) or chromosome sets
95 (polyploidy) are frequent events in various cancers and can drastically affect tumour progression
96 not only through transcriptomic changes but also through the enhancement of CIN itself, creating
97 more and more genetically distinct cancer cell clones.⁸

98 It is believed that the polyploidisation of tumour cells is only a step on the path to
99 aneuploidy.^{21,22} However, polyploid tumour cells can exist without transitioning to aneuploidy.²¹
100 Polyploid tumour cells contribute significantly to cancer progression. Polyploid giant cancer
101 cells (PGCCs) are formed by endoreplication or fusion of several cells and are found in high-
102 grade and chemoresistant cancers, predominantly in breast, ovarian and colorectal cancers.^{23,24}
103 PGCCs can survive anticancer therapy, are extremely tumourigenic and contribute to cancer
104 metastasis.^{23,24} PGCCs and their daughter cells, collectively called tumour buds and located at
105 the invasive front of tumours,²⁵ have a mesenchymal phenotype and a high capacity for invasion
106 through changes in the expression of factors that mediate EMT.^{26–28} In the MDA-MB-231 breast
107 cancer cell line, PGCCs moved more slowly than normal cancer cells, but showed high
108 migratory persistence.²⁹ This migratory phenotype is associated with the dysregulation of the
109 actin network and RhoA–Rho-associated protein kinase (ROCK)1 signalling pathway, which
110 drives increased cell stiffness.²⁹ As shown in LoVo and HCT116 colorectal cancer cells *in vitro*
111 and *in vivo*, the migration and invasion of PGCCs and their daughter cells might be determined

112 by S100A4 and its associated molecular network, potentially involving regulation of the structure
113 and function of the annexin A2–S100A10 complex to influence cathepsin B, as well as
114 cytoskeletal associations with 14-3-3 ζ/δ and ezrin.³⁰ In addition to PGCCs, other polyploid cells
115 can contribute to tumour metastasis. For example, as shown in the DLD-1 cell line, tetraploid
116 tumour cells observed at the invasive front of colorectal adenocarcinomas are characterised by an
117 enhanced capability to migrate and invade.³¹

118 Aneuploidy has long been known to be associated with an increased expression of genes
119 related to EMT, cancer cell migration, invasion and metastasis.³² However, different
120 aneuploidies have distinct effects on cancer cell invasion.³³ For example, DLD-1 colorectal
121 cancer cells with trisomy of chromosome 7 or chromosome 13 invade more actively than diploid
122 cells, both in standard and stressful conditions (hypoxia, etc.) *in vitro*.³⁴ Similarly, trisomy of
123 chromosome 5 enhances the invasive potential of HCT116 colorectal cancer cells *in vitro* and *in*
124 *vivo* through partial EMT and upregulation of matrix metalloproteinases (MMPs).³³ By contrast,
125 trisomy of chromosome 13 or chromosome 18 significantly decreases invasion of HCT116
126 colorectal cancer cells *in vitro*, potentially because of aneuploidy-induced dosage imbalances
127 that may interfere with different cellular functions, including cell motility.³³

128

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

131 Gene fusions are a frequent result of chromosomal rearrangements and can result from
132 translocations, deletions, inversions and duplications, as well as chromothripsis, a catastrophic
133 genomic event leading to massive rearrangements of multiple chromosomes.³⁵ Owing to the
134 large number of gene fusions, their role in cancer cell migration and invasion could be the topic
135 of another review, so we consider here some of the most common gene fusions. The first gene
136 fusion to be discovered, *BCR–ABL*, is the result of a reciprocal translocation between
137 chromosomes 22 and 9, and is detected in more than 96% of patients with chronic myeloid

138 leukaemia.³⁵ This fusion causes alterations in the actin cytoskeleton that promote the motility of
139 chronic myeloid leukaemia cells, as demonstrated in various cell lines *in vitro*.^{36,37} The
140 *TMPRSS2-ERG* gene fusion can arise from the inversion or interstitial deletion of chromosome
141 21q22 and is found in 50% of prostate cancers.³⁵ This gene fusion leads to the overexpression of
142 *ERG* (ETS-related gene), a transcription factor, which, in turn, promotes prostate tumour cell
143 movement through Notch signalling or transcriptional activation of MMP9 and plexin A2, a
144 semaphorin co-receptor.³⁸⁻⁴⁰ *ERG* overexpression as a result of the *TMPRSS2-ERG* gene fusion
145 event has been demonstrated to promote EMT not only by activating TGF- β signalling but also
146 by inducing WNT signalling.^{41,42} Other gene fusions also contribute to EMT. The *MLL-AF9*
147 translocation t(9;11) is found in acute myeloid leukaemia and promotes tumour invasion
148 associated with the transcription factor ZEB1 in a long-term haematopoietic stem-cell-derived
149 mouse model of acute myeloid leukaemia.⁴³ Fusions between the estrogen receptor gene (*ESR1*)
150 and *YAPI* (which encodes Yes1-associated transcriptional regulator) or *PCDH11X* (which
151 encodes the cell adhesion protein protocadherin 11 X-linked) are associated with the induction of
152 EMT and were shown to enhance the motility of T47D breast cancer cells *in vitro* and the
153 metastasis of T47D xenografts.⁴⁴

154 Gene amplifications are frequently occurring events in many cancers and result in
155 overexpression of genes — mainly oncogenes — that confer a growth or survival advantage on
156 cancer cells. Indeed, *ErbB2* gene amplification is one of the most frequent genetic events in
157 breast cancer, resulting in the overexpression of HER2, which promotes cell proliferation
158 predominantly through the activation of the mitogen-activated protein kinase (MAPK) pathway.
159 However, *ErbB2* gene amplification can also induce breast cancer cell migration and invasion
160 through the HER2-mediated activation of the Rho GTPases Rac1 and Cdc42, master regulators
161 of cytoskeletal dynamics.^{45,46} Overexpression of fibroblast growth factor receptor 1 (FGFR1) due
162 to amplification of the corresponding gene *FGFR1* promotes EMT and increases migration and
163 invasion of H1581 NSCLC cells and DMS114 small cell lung cancer cells *in vitro* by

164 upregulating the expression of transcription factor SOX2, one of the core operators of stemness
165 and EMT.⁴⁷ The amplification of wild-type *EGFR* and subsequent activation of the epidermal
166 growth factor receptor (EGFR) contribute to the non-angiogenic invasive growth of glioblastoma
167 in the patient-derived rat xenograft model probably through the induction of EMT and correlate
168 with glioblastoma invasion in patients.⁴⁸

169 Amplification of growth factor receptor genes is not the only way to induce cancer cell
170 invasion and migration. Amplification of chromosome region 11q13, which encompasses genes
171 encoding regulators of the actin cytoskeleton and cell motility (e.g. cortactin, cofilin, p21-
172 activated kinase 1, etc.), occurs in 30–50% of head and neck squamous cell carcinomas
173 (HNSCC).⁴⁹ An *in vitro* study demonstrated that 11q13 amplification promotes the
174 overexpression of cortactin, which binds to and activates the Arp2/3 actin-nucleating complex,
175 leading to the increased migration and invasion of various HNSCC cell lines (UMSCC2,
176 UMSCC19 and MSK921).⁵⁰ By contrast, 11q13 amplification-driven overexpression of the
177 *PPFIA1* gene, which encodes liprin- α 1, a protein potentially involved in cell–matrix interactions,
178 suppresses migration and invasion of FaDu HNSCC cells *in vitro*.⁵¹ These results indicate the
179 presence of both positive and negative regulators of cell motility in this chromosomal region.
180 Amplification of another chromosome region, 11q22.1–q22.2, is often found in oral squamous
181 cell carcinomas and is associated with lymph node metastasis. This amplification leads to
182 overexpression of the *BIRC3* gene, the protein product of which — cellular inhibitor of apoptosis
183 (cIAP)2 — enhances the migration and invasion of SCC29B oral squamous carcinoma cells *in*
184 *vitro*.⁵²

185 Additional studies have shown that amplification of chromosome regions harbouring non-
186 coding RNAs also triggers tumour cell migration and invasion. Gene-amplification-driven long
187 non-coding RNA (lncRNA) SNHG17 promotes the migration of A549 and PC-9 NSCLC cells *in*
188 *vitro*,⁵³ whereas amplification of lncRNA PCAT6 is important for motility in HepG2 and
189 SMMC-7721 hepatocellular carcinoma cells *in vitro*.⁵⁴ Amplification and subsequent

190 overexpression of miR-151 directly targets RhoGDIA, a putative metastasis suppressor, to
191 promote the migration and invasion of Huh-7 and SMMC-7721 hepatocellular carcinoma cells *in*
192 *vitro* and the metastasis of SMMC-7721 cells.⁵⁵ MiR-182, a member of the miRNA cluster in the
193 chromosomal locus 7q31–34 that is frequently amplified in melanoma, stimulates the migration
194 of SK-MEL-19 melanoma cells *in vitro* and increases the metastatic potential of B16F10 mouse
195 melanoma cells.⁵⁶

196

197 **Gene alterations**

198 In addition to harbouring chromosomal abnormalities, different cancers also contain an
199 abundance of point mutations as well as gene insertions and deletions (indels). These gene
200 alterations play a significant role in various stages of cancer metastasis, and invasion is no
201 exception.⁵⁷ Below, we outline those genes whose alteration affects the migration and invasion
202 of tumour cells; they are divided into several groups, depending on their primary function (Fig.
203 3).

204

205 ***Genes involved in genome maintenance.*** Genes involved in maintaining genome stability are
206 often mutated in cancer. Not only do loss-of-function (LOF) mutations of these tumour
207 suppressors contribute to the acquisition of a mutator phenotype by tumour cells, but they can
208 also affect cancer cell migration and invasion. Mutations in *BRCA1* lead to dysregulation of the
209 Ubc9/caveolin-1/vascular endothelial growth factor (VEGF)/SIRT1/estrogen receptor (ER)- α
210 axis, promote EMT and trigger the migration of HCC1937 triple-negative breast cancer cells *in*
211 *vitro*.^{58,59} The *STAG2* gene, the protein product of which regulates centromere cohesion, is often
212 mutated in various cancers. Most *STAG2* mutations are truncating and, as shown in the U2OS
213 osteosarcoma cell line, the loss of this gene leads to increased EMT-associated tumour cell
214 migration *in vitro*, coincident with decreased expression of E-cadherin and increased expression
215 of N-cadherin.⁶⁰

216 The best known ‘stabiliser’ of the genome and tumour suppressor, however, is p53. *TP53* is
217 often mutated in a wide variety of tumours, from carcinomas and sarcomas to lymphomas and
218 leukaemias.⁶¹ Loss of p53 due to LOF mutations often leads to increased activity of the
219 transcription factors Snail and Twist1, decreased expression of E-cadherin and induction of
220 EMT.^{62–64} In addition, p53 loss activates Rho GTPases to increase cell migration, as shown in
221 mouse embryonic fibroblasts and A375P melanoma cells *in vitro*.^{65,66} However, loss of *TP53*
222 might not always be sufficient to promote tumour cell invasion and metastasis, as shown *in vivo*
223 in PVTT-1 hepatocellular carcinoma xenografts and transgenic mouse rhabdomyosarcoma
224 model, indicating that gain-of-function (GOF) mutations of this gene are more potent activators
225 of the metastatic cascade.^{64,67}

226 GOF mutations in *TP53* cause an even more prominent effect on tumour cell invasiveness
227 than do LOF mutations.^{68,69} Driver *TP53* GOF mutations often occur at codons 175, 248 and
228 273⁶¹ and endow the p53 protein with new abilities to regulate hundreds of different genes
229 including other tumour suppressors.⁷⁰ The mutants p53 R175H and R273H have been shown to
230 bind to and inactivate the tumour suppressor p63 to form a mutant p53–p63 complex.⁶ This
231 mutant complex suppresses Split and Hairy-related protein 1 (Sharp-1, a metastasis suppressor)
232 and cyclin G2 and enhances TGF- β -mediated invasion and metastasis of MDA-MB-231 breast
233 cancer cells *in vitro* and *in vivo*,⁷¹ as well as accelerating integrin recycling and activating
234 signalling by the receptor tyrosine kinases EGFR and Met via Rab-coupling protein (RCP) in
235 H1299 lung and MDA-MB-231 breast cancer cells.^{72,73} In these cancers, mutant p53 also
236 promotes EGFR and Met signalling through the inactivation of a suppressor of invasion, Dicer
237 ribonuclease,⁷⁴ and enhances integrin and EGFR recycling and focal adhesion turnover by
238 modulating components of the endosomal machinery.⁷⁵ Inactivation of p63 by p53 mutants can
239 also alter the expression of miRNAs involved in tumour cell migration. For example, mutant-
240 p53-mediated upregulation of miR-155 leads to the increased migration and invasion of ZR-75-1
241 breast and H1299 lung cancer cells *in vitro*,⁷⁶ and downregulation of tumour suppressor

242 microRNA let-7i induced by the mutant p53–p63 complex leads to enhanced invasion of H1299
243 lung cancer cells *in vitro*.⁷⁷ As demonstrated in H1299 lung cancer cells *in vitro*, formation of the
244 mutant p53–p63 complex and the associated increase in cancer cell migration and invasion can
245 be inhibited by the activating transcription factor 3 (ATF3) protein, which binds the mutant
246 forms of p53 and thus facilitates p63 activation.^{78,79} It is important to note that the mutant p53–
247 p63 complex and the mechanisms described above are not always required for the migration and
248 invasion of tumour cells. Inactivation of Dicer ribonuclease mediated by mutant p53 can occur
249 independently of the formation of the mutant p53–p63 complex.⁷⁴

250 In addition, GOF mutant forms of p53 can trigger EMT via overexpression of Twist,⁸⁰
251 stabilisation of Slug⁸¹ and also by acting on ZEB1.⁸² Mutant p53 can enhance the expression of
252 the A1AT protein, which promotes EMT-associated migration and invasion of H2009 lung
253 cancer cells *in vitro* and drives invasion of H2009 cells in the chick chorioallantoic membrane *in*
254 *vivo* assay.⁸³ The p53 R248Q mutant activates the phosphorylation of Stat3, which results in the
255 enhanced EMT-dependent migration of HCT116 colorectal cancer cells and H1299 NSCLC cells
256 *in vitro*.⁸⁴ Mice with p53 mutations in addition to the loss of another tumour suppressor, RB1,
257 develop mammary tumours with EMT features.⁸⁵

258 Numerous other studies have demonstrated the effect of GOF p53 mutations on a multitude
259 of cell locomotion regulators.⁶⁹ It should be noted, however, that p53 mutants can impact cell
260 movement negatively as well as positively. For example, dominant-negative p53 mutants, such
261 as R175H, R273H, R280K, and R249S — can induce varying degrees of invasive potential in
262 combination with the wild-type form of p53 in hTERT-HME1 (non-malignant) immortalised
263 epithelial mammary cells. Thus, each of these p53 mutants may specifically affect the metastatic
264 ability of cancer cells.⁸⁶ In contrast, the p53 R248Q mutant negatively affects the migration of
265 MDA-MB-231 breast and H1299 lung cancer cells *in vitro* and alters the distribution of MDA-
266 MB-231 cells injected into zebrafish embryos, and contributes to mesenchymal–epithelial

267 transition (the opposite of EMT).⁸⁷ More research is therefore needed to understand the effects of
268 different p53 GOF mutations on tumour cell motility and invasiveness.

269

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

274 A significant effect on cancer cell migration and invasion is exerted by alterations in the genes
275 encoding various growth factor receptors. In addition to the amplification of genes encoding
276 various growth factor receptors (described above), point mutations and indels in these genes can
277 also affect the motility of tumour cells. The *EGFR* L858R mutation enhances the migration and
278 invasion of A549, H1299 and CL1-0 lung cancer cells *in vitro*.^{88,89} Notably, however, HOG
279 glioma cells with this mutation migrate slower *in vitro* than cells with wild-type *EGFR*.
280 Probably, this is due to the fact that *EGFR* oncogene does not initially provide a selective
281 advantage for HOG cells while the *EGFR* mutation negatively affects cell growth and
282 migration.⁹⁰ Another mutant, EGFRvIII, is characterised by the loss of two extracellular domains
283 owing to the deletion of exons 2–7, which renders the mutant receptor constitutively active and
284 unable to bind ligands. EGFRvIII promotes the migration and invasion of glioblastoma cells
285 through the induction of proteases, integrin signalling and other mechanisms.^{91–93} The so-called
286 ‘gatekeeper’ V561M mutation in *FGFR1* confers resistance to FGFR inhibitors, as well as
287 promoting the mesenchymal phenotype and enhancing the ability of H1581 NSCLC cells to
288 migrate and invade *in vitro*.⁹⁴ Activating mutations in *FGFR2* contribute to a loss of polarity and
289 impair directional cell migration but promote invasion of HEK-293FT endometrial cancer cells
290 *in vitro*.⁹⁵

291 Mutations in Ras-family GTPases are very common in various cancers and significantly
292 affect tumour progression.⁹⁶ *HRAS* Q61R and *NRAS* Q61R driver mutations induce EMT and

293 enhance the migration of Nthy-ori 3-1 thyroid cancer cells and MCF10A breast epithelial cells,
294 respectively.^{97,98} Driver mutations in *KRAS* at position G12 promote EMT via Wnt/ β -catenin and
295 TGF- β signalling pathways in the iKAP mouse model of colorectal cancer *in vivo*⁹⁹ and in
296 various pancreatic cancer cell lines *in vitro* and *in vivo*.^{100,101} Moreover, the *KRAS* G12 and
297 *HRAS* G12 mutants can modulate the function of the Rho GTPases RhoA, Rac1 and Cdc42
298 through the Ras and PI3K signalling pathways in the Caco-2 colorectal cancer cell line *in vitro*
299 and thereby mediate migration and invasion.¹⁰² Overexpression of *KRAS* G12V leads to a
300 decrease of collective invasion of MCF10A cells.¹⁰³

301 Mutations in genes encoding downstream effectors of Ras GTPases also affect the ability
302 of tumour cells to move. The *BRAF* V600E driver mutation occurs in almost half of all
303 melanoma cases and enhances the kinase activity of the BRAF protein.¹⁰⁴ The V600E mutation
304 induces the migration and invasion of WM3211 melanoma cells *in vitro* and the invasion of
305 mouse melanoma *in vivo* by stimulating integrin signalling, actin protrusion formation and the
306 expression of MMPs through activation of extracellular signal-regulated kinase (ERK)/
307 MAPK.¹⁰⁵ The *BRAF* V600E mutant also contributes to invasion of cancers other than
308 melanoma. In thyroid cancer, the *BRAF* V600E mutant promotes cell movement through the
309 nuclear factor (NF)- κ B pathway as demonstrated in WRO and KTC-3 cell lines *in vitro*¹⁰⁶, or by
310 mediating hypomethylation and subsequent overexpression of the gene encoding WAS/WASL
311 Interacting Protein Family Member 1 (WIPF1), as demonstrated in K1, OCUT1 and FTC133
312 cells *in vitro* and K1 cells *in vivo*.¹⁰⁷ In the Caco-2 colorectal cancer cell line, *BRAF* V600E
313 represses E-cadherin and enhances the activity of Rho GTPases.¹⁰² Other evidence also supports
314 a role for *BRAF* mutants in EMT-associated tumour invasion.^{108,109}

315 Mutations in the genes encoding ERK/MAPKs or MAPK/ERK kinases (MEKs) also
316 modulate tumour cell movement. The *ERK3* L290P/V mutation promotes the migration and
317 invasion but not proliferation of H1299 and A549 NSCLC cells *in vitro*.¹¹⁰ Loss of MKK4
318 protein due to *MAP2K4* LOF mutations enhances the invasion associated with peroxisome

319 proliferator-activated receptor γ (PPAR γ) of various lung cancer cell lines (344SQ, 393P and
320 H2009) *in vitro*.¹¹¹

321 *PIK3CA* and *PTEN*, which encode components of the PI3K signalling pathway, are among
322 the most frequently mutated genes in various cancers.¹¹² E545K and H1047R mutations in the
323 p110 catalytic subunit of PI3K, which confer constitutive activity, have been shown to promote
324 the migration and invasion of colorectal,¹¹³ gastric,¹¹⁴ cervical¹¹⁵ and breast cancer¹¹⁶ and
325 HNSCC cells.¹¹⁷ In NOK and EPC1 HNSCC cell lines, the expression of mutant *PIK3CA*
326 together with the downregulation of p120 catenin induces tumour invasion *in vitro*, including in
327 3D organotypic cultures, through an increase in the expression of MMPs.¹¹⁸ *PTEN* LOF
328 mutations are observed in various cancers¹¹⁹ and contribute to EMT and the dissemination of
329 tumour cells.^{120,121} For example, deletion of *PTEN* leads to increased collective invasion of
330 MCF10A cells in contrast to *KRAS* G12V overexpression as mentioned above. Interestingly, the
331 double *PTEN* and *KRAS* mutant cells show decreased collective behaviour, suggesting that
332 *KRAS* dominates the collective migration phenotype.¹⁰³ GOF mutations in *PTEN* are also known
333 to modulate tumour cell movement. For example, the A126G mutant promotes the migration of
334 PC-3 prostate cancer cells *in vitro*.¹²²

335 Mutations in the genes encoding AKT and mammalian target of rapamycin (mTOR),
336 which are involved in the PI3K signalling pathway, are rare in cancers.¹²³ However, mutant
337 forms of these proteins can still contribute to cancer cell migration and invasion. The *AKT1*
338 E17K mutation (0.6–2% frequency in NSCLC) enhances the migration and invasion of normal
339 lung epithelial cells (BEAS-2B) by relocating the cyclin-dependent kinase inhibitor p27 into the
340 cytoplasm from the nucleus and inhibiting RhoA signalling.¹²⁴ The same mutated form of *AKT1*
341 increases the migration and invasion of human mammary luminal (HMLER) but not
342 myoepithelial (BPLER) cells.¹²⁵ GOF mutations conferred by mutated *mTOR* occur with a
343 frequency of no more than 1% for various types of cancer; some of these mutations (e.g.
344 A1256G and G7076A) promote cell migration and invasion *in vitro*.¹²⁶

345 Mutations in other genes implicated in cell survival have also been reported to influence
346 cell invasion. Retinoblastoma protein, encoded by *RBI*, is a well-known tumour suppressor that
347 plays a role in controlling cell cycle progression.¹²⁷ Different mechanisms are involved in *RBI*
348 loss, including LOF mutations and deletions.¹²⁷ The knockdown-mediated loss of *RBI*
349 expression in PC3, PC3-ML and LNCaP prostate cancer cells leads to the acquisition of an
350 increased migratory and invasive capacity with decreased expression of E-cadherin *in vitro*.¹²⁸
351 The loss of *RBI* in MYC-overexpressing mouse mammary epithelial cells promotes invasion *in*
352 *vitro* and enhances the invasive phenotype in MYC-overexpressing xenograft tumours.¹²⁹
353 Moreover, *RBI* suppression was demonstrated to stimulate collective invasion rather than single-
354 cell invasion of basal-like breast carcinoma cells *in vitro* and *in vivo*. Importantly, Rb
355 knockdown also induced expression of CD44, lymphovascular invasion, the release of
356 circulating tumor cells, and distant metastasis.¹³⁰ The *CAVI* gene encodes caveolin-1, a
357 component of caveolae — specialised plasma membrane invaginations that regulate cell
358 proliferation and migration.¹³¹ Using the highly metastatic Met-1 mammary epithelial cell line, it
359 was demonstrated the *CAVI* P132L mutation, which occurs in 16% of breast cancers, promotes
360 migration and invasion, and activates various signalling pathways involved in metastasis.¹³² The
361 tyrosine phosphatase SHP2 (*PTPN11*) transmits signals from tyrosine kinase receptors and
362 regulates cell proliferation. A GOF mutation in *PTPN11* that confers a D61G substitution
363 enhances the migration and invasion of MDA-MB-231 and MCF-7 breast cancer cells *in vitro*
364 and the metastasis of both cell lines *in vivo* through the activation of the Ras and PI3K signalling
365 pathways.¹³³ Caspases are best known as essential mediators of the apoptotic program and cell
366 survival, but mutations in the *CASP8* gene have been shown to accelerate migration and invasion
367 of UM-SCC-47 HNSCC cells *in vitro* and their growth *in vivo*.¹³⁴ Probably, it can be related to
368 the catalytic and noncatalytic modes of action by which *CASP8* influences cell adhesion and
369 migration.¹³⁵
370

371 *Actin cytoskeleton regulators.* As mentioned above, Rho GTPases are key regulators of
372 actin cytoskeleton remodelling. The best-studied Rho GTPases — Rac1 and RhoA — are often
373 mutated in various types of cancer.¹³⁶ *RAC1* is the third most frequently mutated gene in
374 melanoma after *BRAF* and *NRAS*.¹³⁷ The *RAC1* P29S driver mutation, which results from a C>T
375 transition in response to UV damage, generates a more active form of Rac1. This mutant form is
376 characterised by increased switching from the inactive, GDP-bound to the active, GTP-bound
377 state, which enhances the interaction of Rac1 with its downstream effectors.¹³⁸ The *RAC1* P29S
378 mutant promotes the migration of melanocytes¹³⁹ and invasion of mouse embryonic fibroblasts
379 *in vitro*.¹⁴⁰ Although melanoma cells (104T cell line) with the *RAC1* P29S mutation form
380 lamellipodia more actively, this mutant negatively affects the formation of invadopodia and
381 invadopodia-dependent matrix degradation *in vitro*. This can indicate that *RAC1* P29S-harboring
382 melanoma cells have an enhanced migration, but attenuated invasion.¹⁴¹ *RHOA* is a driver gene
383 in many cancers, such as T-cell lymphoma and gastric cancer.¹⁴² LOF mutants of *RHOA* (G17E,
384 Y42C and Y42S) that are present in diffuse-type stomach cancers lead to the inactivation of
385 RhoA–ROCK1 signalling and increased migration of MKN74 gastric tubular adenocarcinoma
386 cells *in vitro*.¹⁴³ Moreover, as shown in the orthotopic xenograft mouse model, MKN74 gastric
387 cancer cells with *RHOA* mutations are more invasive and acquire immune resistance.¹⁴⁴

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

392 The activity of Rho GTPases is positively regulated by Rho guanine nucleotide-exchange
393 factors (GEFs) and negatively by Rho GTPase-activating proteins (GAPs);¹⁴⁵ consequently,
394 mutations in the genes encoding these Rho GTPase regulators significantly affect the migration
395 and invasion of tumour cells. The *PREX2* gene, which encodes a RhoGEF, is often mutated in
396 metastatic solid tumours.¹⁴⁶ The *PREX2* S1113R mutant protein, present in patients with

397 hepatocellular carcinoma, has been shown to promote the migration of Huh7 liver tumour cells
398 *in vitro*.¹⁴⁷ *RGS7*, which encodes a Rho GTPase-activating protein, is a tumour suppressor that is
399 mutated in melanoma. The *RGS7* R44C mutation destabilises the protein, which thereby results
400 in the enhanced motility of A375 melanoma cells *in vitro*.¹⁴⁸ *ARHGAP35*, which encodes a
401 negative regulator of Rho GTPases, is mutated in 15% of endometrial tumours. *ARHGAP35*
402 GOF mutations (S866F and Δ 865–870) contribute to random MDA-MB-231 breast cancer cell
403 migration *in vitro*, which might promote the exploratory behaviour of tumour cells.¹⁴⁹

404 Rho GTPases regulate downstream signalling effectors such as ROCKs, p21-activated
405 kinases (PAKs), the SCAR/WAVE complex, LIM kinase (LIMK), cofilin and Arp2/3, which
406 control actin cytoskeleton remodelling. Despite these effectors rarely being mutated in various
407 cancers, it is logical to assume that mutations in their encoding genes, if they do occur, might
408 affect the migration and invasion of tumour cells. Loss of the *ABII* gene (which encodes a
409 component of the SCAR/WAVE complex) leads to the induction of EMT and increased
410 migration and invasion of RWPE-1 benign prostate epithelial cells in 2D and 3D *in vitro*
411 systems.¹⁵⁰ However, these results contradict the general consensus that overexpression of the
412 SCAR/WAVE complex is associated with increased cancer invasion and poor prognosis, as
413 outlined by Molinie and Gautreau.¹⁵¹ The E329K mutant of *PAK4* promotes the motility of PC3
414 prostate carcinoma cells *in vitro*,¹⁵² and GOF mutations in the *ROCK1* gene promote mouse
415 embryonic fibroblast migration *in vitro*.¹⁵³ However, it is important to note that, as mentioned
416 above, mutations in downstream effectors of Rho GTPases are rare in cancer, and the
417 dysregulation of these effectors in tumour cells is predominantly caused by other mechanisms.¹⁵⁴

418

419 ***Genes involved in cell adhesion and ECM proteolysis.*** Changes in cell adhesion and proteolysis
420 of the ECM are inextricably linked to cell movement.¹⁵⁵ Again, the genes underlying these
421 processes are rarely mutated in cancers; however, experimental data indicate the importance of
422 their potential mutation in the movement of tumour cells.

423 Integrins play a big role in cell adhesion, and changes in their expression promote cancer
424 invasion.¹⁵⁵ Although integrins are frequently dysregulated in various types of cancer, integrin
425 mutations are poorly studied, especially in terms of their effect on tumour cell migration.¹⁵⁶ The
426 integrin $\beta 1$ mutant T188I, which is found in poorly differentiated human squamous cell
427 carcinoma of the tongue, enhances cell spreading (anchoring to the substrate) and actin
428 cytoskeleton assembly, but does not promote migration or invasion of mouse keratinocytes *in*
429 *vitro*.^{157,158} Note, cell spreading and cell motility are mechanistically different phenomena
430 despite outward similarities.¹⁵⁹ Integrin $\alpha 7$ is frequently inactivated in prostate tumours and
431 leiomyosarcoma due to truncating mutations in the corresponding gene, and expression of wild-
432 type *ITGA7* inhibits the migration of prostate cancer (PC-3 and Du145) and SK-UT-1
433 leiomyosarcoma cells *in vitro*.¹⁶⁰ Nevertheless, the effect of most integrin mutations on tumour
434 cell migration and invasion remains unstudied.

435 Mutations in the genes encoding α -catenin (*CTNNA2* and *CTNNA3*) are characteristic of
436 laryngeal squamous cell carcinoma and have been shown to promote tumour invasion of SCC-2
437 oral cancer cells *in vitro*.¹⁶¹ The adaptor protein paxillin (encoded by the *PXN* gene), a key
438 component of focal adhesions, was mutated in up to 9.4% of NSCLC cases analysed by
439 Jagadeeswaran *et al.*¹⁶² The most frequent mutation, A127T, enhances focal adhesion and
440 lamellipodia formation in HEK-293 human embryonic kidney cells *in vitro*¹⁶³ and promotes the
441 invasion of H522 NSCLC cells *in vivo*.¹⁶² EPHB6 is a receptor for ephrin-B ligands that
442 modulates cell adhesion and migration. The *EPHB6* Q926R mutation activates RhoA through the
443 induction, via JNK signalling, of cadherin-11 expression and increases the invasion of A549
444 lung, Huh7 liver and A375P skin cancer cells *in vitro*.¹⁶⁴ The deletion of exon 33 in the gene
445 encoding focal adhesion kinase (*FAK*) confers a gain of function on the protein that enhances
446 migration and invasion of MDA-MB-468 breast cancer cells *in vitro*.¹⁶⁵ Onder *et al.* showed that
447 truncating mutations in the *CDHI* gene, that lead to the expression of a dominant-negative
448 protein, promote cell migration and growth of HMLER cells *in vitro* and *in vivo*, but to a lesser

449 extent than the shRNA-mediated loss of E-cadherin .¹⁶⁶ Other studies showed that *CDH1*
450 mutations do not affect EMT or the motility of various breast cancer cell lines (MDA-MB-231,
451 MCF-7, etc.) *in vitro*.^{167,168} All these data might indicate the cell-specific effect of *CDH1*
452 mutations.

453 Tumour cells must be able to degrade the ECM in order to penetrate the surrounding tissue
454 and disseminate. It is therefore logical to assume that mutations in genes encoding proteases
455 might alter the invasive potential of tumour cells. Similar to the situation regarding Rho GTPase
456 effectors and integrins, most of the genes encoding various proteases, especially MMPs, are
457 infrequently mutated in cancers; however, there are some data regarding the impact of their
458 alterations on cancer cell migration and invasion. For example, mutations in the *MMP8* gene,
459 often found in melanoma, enhance the migration of immortalised transformed human Mel-STR
460 melanocytes *in vitro* and *in vivo*. Surprisingly, wild-type *MMP8* inhibits melanoma cell
461 migration.¹⁶⁹ Migration and invasion-suppressive role of *MMP8* are also known in oral tongue
462 squamous cell and breast carcinomas.^{170,171} Moreover, in breast cancer, *MMP8* can prevent
463 metastasis formation.¹⁷¹ The exact mechanisms of the suppressive effects of *MMP8* are still
464 unclear. Probably, *MMP8* triggers migration- and invasion-suppressive molecular cascades
465 through cleavage of various non-ECM substrates with specific regulatory functions.¹⁷² Similarly,
466 mutations in the gene encoding a disintegrin-like and metalloproteinase domain with
467 thrombospondin type 1 motifs (*ADAMTS18*) are potential drivers of melanoma and promote the
468 migration of A375 melanoma cells *in vitro* and the metastasis of Mel-STR cells *in vivo*.¹⁷³
469 Notably, however, evidence exists that mutations in protease genes can confer an inhibitory
470 effect on the movement of tumour cells. Mutant forms of *ADAMTS16* have been shown to
471 inhibit the motility of A2780CP20 ovarian cancer cells *in vitro* and *in vivo*.¹⁷⁴ Breast cancer-
472 associated mutations in the *ADAM12* gene interfere with the intracellular trafficking of the
473 corresponding protein and inhibit the migration of mouse embryonic fibroblasts *in vitro*.¹⁷⁵ In
474 general, proteases (especially MMPs) are considered as potential druggable targets in anti-cancer

475 therapy,^{176,177} but whether their mutants can be therapeutically targeted is currently unknown,
476 probably due to the fact that these genes are very rarely mutated in cancers. Furthermore, the
477 enhanced migration of MMP8 mutant immortalized melanocytes emphasises the need to assess
478 the function of each MMP individually to define its precise role in cancer.

479

480 ***EMT regulators.*** As demonstrated above, mutant forms of many oncogenes and tumour
481 suppressors can modulate EMT through different mechanisms. But what about other regulators
482 of EMT? Although mutations in genes encoding transcription factors that are involved in EMT
483 (Twist, Snail, Slug, and Zeb1) are known to be extremely rare in cancer,¹⁷⁸ the activity of these
484 transcription factors is regulated by other genes, mutations in which can occur more frequently in
485 various cancers. For example, mutations in the driver genes (*ADPGK* (encodes ADP-dependent
486 glucokinase), *PCGF6* (polycomb group RING finger protein 6), *PKP2* (plakophilin 2), *NUP93*
487 (nucleoporin 93) and *SLC22A5* (solute carrier family 22 member 5)) can affect EMT and
488 promote MDA-MB-231 breast cancer cell migration *in vitro*.¹⁷⁹ The gene encoding another EMT
489 regulator, TRIM21, which promotes the proteasomal degradation of Snail and thereby suppresses
490 migration and invasion, is rarely mutated in breast cancer (frequency < 1%), but the R64Q
491 mutation abrogates the ability of TRIM21 to mediate Snail degradation and thus promotes breast
492 cancer cell invasion.¹⁸⁰ GOF mutations in the TGF- β receptor II gene (*TGFBR2*) induce the
493 relocalisation of E-cadherin from the cell membrane to the cytoplasm and overexpression of
494 vimentin and promote TGF- β signalling, migration and invasion of HSC-2 oral squamous cell
495 carcinoma cells *in vitro*, contributing to aggressive cancer behaviour.¹⁸¹ Mutations in the genes
496 that encode Smad transcription factor proteins, which are key mediators of TGF- β signalling, can
497 promote TGF- β -mediated EMT.^{182,183} Furthermore, driver mutations in the *APC*, *CTNNB1* and
498 *NOTCH1* genes, and other components of the WNT and Notch signalling pathways, contribute to
499 EMT in various cancers.^{184–186}

500

501 **Miscellaneous genes.** As a consequence of mutation, genes that are not directly related to the
502 regulation of cell movement can sometimes acquire new functions and thus promote cancer cell
503 migration and invasion. Missense and nonsense mutations in the mitochondrial gene *ND6*, which
504 normally encodes a subunit of NADH dehydrogenase (ubiquinone), promote migration and
505 invasion of A549 lung adenocarcinoma cells *in vitro*, probably via the increased generation of
506 reactive oxygen species.¹⁸⁷ Activating mutations in the *GRM3* gene, which encodes a G-protein-
507 coupled receptor, occur in melanoma and stimulate the migration of A375 melanoma cells *in*
508 *vitro*, probably through phosphorylation of MEK.¹⁸⁸

509

510 **Studying the effect of genetic alterations on tumour cell movement**

511 Most current studies focus on the investigation of the effects of changes in various
512 epigenetic determinants and gene expression on tumour cell migration and invasion while the
513 impact of genetic alterations on the ability of tumour cells to move undeservedly remains poorly-
514 studied. However, the irreversible nature of these genetic alterations might actually contribute
515 more significantly to the invasion of tumour cells than other factors do.

516

517 **Current challenges.** Many of the mutations described above occur in genes that regulate a wide
518 array of cellular processes, and it is often difficult to separate their impact on migration and
519 invasion from their influence on tumour formation — this can be a serious obstacle in studying
520 the effect of genetic alterations on the motility of tumour cells. Moreover, it is hard to conclude
521 whether tumour cell movement hinges upon certain mutations or other, non-genetic triggers.
522 Another important issue is the need to identify mutational drivers of invasion and metastasis,
523 both universal and specific for different types of cancer. Analysis of the studies discussed in this
524 review shows that some genes (*TP53*, *EGFR*, *PIK3CA*, etc.) can be common for various cancers
525 in terms of the effect of their mutations on tumour cell migration and invasion, whereas other
526 genes are strongly specific for certain malignant tumours: for example, *RAC1* and *ADAMTS18* in

527 melanoma, and *APC* in colorectal cancer (see Table 1). Even though some genes that are
528 involved in cell motility are rarely mutated in cancers (such as downstream effectors of Rho
529 GTPases and integrins), their mutations, no matter how infrequently they occur, might play a big
530 role in driving cancer invasion. Moreover, each cancer is likely to be unique in its genetic
531 landscape, and therefore mutational drivers important for invasion could vary significantly from
532 tumour to tumour. Thus, further studies should be focused on the development of an atlas of
533 mutational drivers of cancer invasion as an important step towards understanding the genetic
534 subtleties that underlie tumour dissemination.

535

536 ***Approaches to analysing mutational drivers of cancer invasion.*** Different approaches can be
537 used to identify and study mutational drivers of cancer invasion. Metastatic mouse models of
538 various cancers are an effective way to identify genetic alterations that contribute to tumour cell
539 migration, invasion and metastasis.^{189–192} A 2017 study used a metastatic model of colorectal
540 cancer to demonstrate that pronounced migration of tumour cells depends on the combined effect
541 of mutations in *APC*, *KRAS*, *TP53* and *SMAD4*.¹⁹³ It seems reasonable to analyse cancer
542 genomes by focusing on the functionally significant mutations in genes that regulate critical
543 processes in cell migration and invasion — for example, EMT, actin cytoskeleton remodelling,
544 proteolysis, and so on — and to validate their significance *in vitro* and *in vivo*. Another potential
545 approach is to analyse the mutational landscape of tumour cells located in the invasive front and
546 to select for genetic alterations that are not present in the tumour core. For example, local
547 invasion is a hallmark of malignant gliomas, making glioma cells a candidate model for finding
548 drivers of cancer invasion.¹⁹⁴ However, data also indicate that highly dynamic cells are present
549 not only at tumour borders but also in the tumour core, as was demonstrated in *NICD/p53^{-/-}*
550 mouse intestinal cancer¹⁹⁵ and orthotopic human glioblastoma model¹⁹⁶, which significantly
551 reduces the chance of finding mutations that drive cancer invasion when comparing the invasive
552 front to the tumour core. In this case, it therefore seems reasonable to compare the mutational

553 landscape of invasive and non-invasive tumour cells within the same tumour. Specific molecular
554 markers could potentially be used to distinguish motile tumour cells from non-motile tumour
555 cells in the primary tumour, and meticulous examination of the genomic landscape of such cells
556 could uncover novel mutational drivers of cancer invasion. However, no effective and reliable
557 markers to help identify truly motile tumour cells currently exist.¹⁹⁷

558 In our studies, we have shown that the intratumoural morphological heterogeneity of
559 invasive ductal carcinoma of the breast (now classified as invasive carcinoma of no special type)
560 is a reflection of various patterns of tumour cell invasion. In particular, breast cancer cells can
561 exist as single entities or be arranged in either small groups (2–5 cells) or multicellular
562 structures: tubular, alveolar, solid, trabecular and torpedo-like structures (Fig. 4).^{198,199} Tubular
563 and alveolar structures are transcriptionally similar and demonstrate a similar expression of
564 epithelial and mesenchymal markers. Solid structures show an increase in mesenchymal traits
565 but retain epithelial features. Trabecular structures, small groups of tumour cells and single
566 tumour cells all display a pronounced mesenchymal phenotype and a dramatic decrease in
567 epithelial traits as well as significant enrichment of cancer invasion signaling pathways.¹⁹⁸ The
568 presence of alveolar and trabecular structures in breast tumours is associated with increased
569 lymph node metastasis^{200,201} and distant recurrence in patients treated with neoadjuvant
570 chemotherapy.²⁰² Distant metastases are also frequently detected in breast cancers with single
571 tumour cells with epithelial-like morphology,²⁰³ and in breast cancers that express kinesin-14
572 (KIF14) and mitochondria-eating protein (Mieap) but lack ezrin (EZR) at the tips of torpedo-like
573 structures.¹⁹⁹ The nature of torpedo-like structures, e.g. their EMT features, remains to be
574 elucidated; however, KIF14, Mieap, and EZR proteins are known to be important regulators of
575 tumour cell migration and invasion.^{204–206} Based on all these results, we assumed that tubular and
576 alveolar structures show decreased invasive potential, whereas solid, trabecular and torpedo-like
577 structures, as well as small groups of tumour cells and single tumour cells, are highly invasive.
578 The intratumoural morphological heterogeneity of breast cancer is therefore an attractive model

579 for detecting mutational drivers of tumour cell invasion — for example, by comparing the
580 genomic landscapes of highly invasive and less invasive morphological structures. Moreover,
581 comparative analysis of multicellular structures (e.g. solid, trabecular or torpedo-like structures)
582 against single tumour cells might provide information regarding genetic mutations that are
583 involved in collective and individual modes of cancer invasion.

584

585 **Conclusions and discussion**

586 Different chromosomal and gene aberrations influence cancer cell migration and invasion.
587 CIN affects cancer cell movement through mechanisms associated with polyploidy and
588 aneuploidy, as well as with gene fusion and amplification. Gene alterations trigger or suppress
589 the spread of cancer cells in several ways, by influencing genes that affect genome maintenance,
590 cell survival, actin cytoskeleton remodelling, EMT, adhesion and proteolysis. Such genetic
591 drivers are of particular interest as potential prognostic markers and targets for anti-metastatic
592 therapy.

593 Indeed, some of the mutational drivers discussed in this review have already been
594 established as potential targets for anticancer therapy – p53 hotspot mutations,²⁰⁷ *EGFR*
595 mutations²⁰⁸ and PI3K p110 α E545K and H1047R mutants.²⁰⁹ The main objective of anticancer
596 therapy is to stop tumour growth and to kill cancer cells. However, another therapeutic approach,
597 which is receiving ever-increasing interest, is to block the ability of tumour cells to invade and
598 metastasise. Migrastatics are a novel class of anticancer drugs aimed at attenuating cancer cell
599 migration by targeting the signalling pathways and downstream effectors that are involved in cell
600 motility.²¹⁰ The downside of these therapeutics is that they can be toxic for all types of moving
601 cell — for example, fibroblasts, keratinocytes and leukocytes.²¹¹ In this regard, mutational
602 drivers of cancer invasion could constitute especially interesting targets for migrastatics as these
603 genetic alterations are present only in tumour cells. Nevertheless, this issue requires a great deal
604 of further research.

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

616

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621

622 **AUTHOR CONTRIBUTIONS**

623 N.M.N and S.Y.Z wrote the manuscript. A.M.G. and E.V.D. supervised, proofread and
624 provided input on the manuscript.

625

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633

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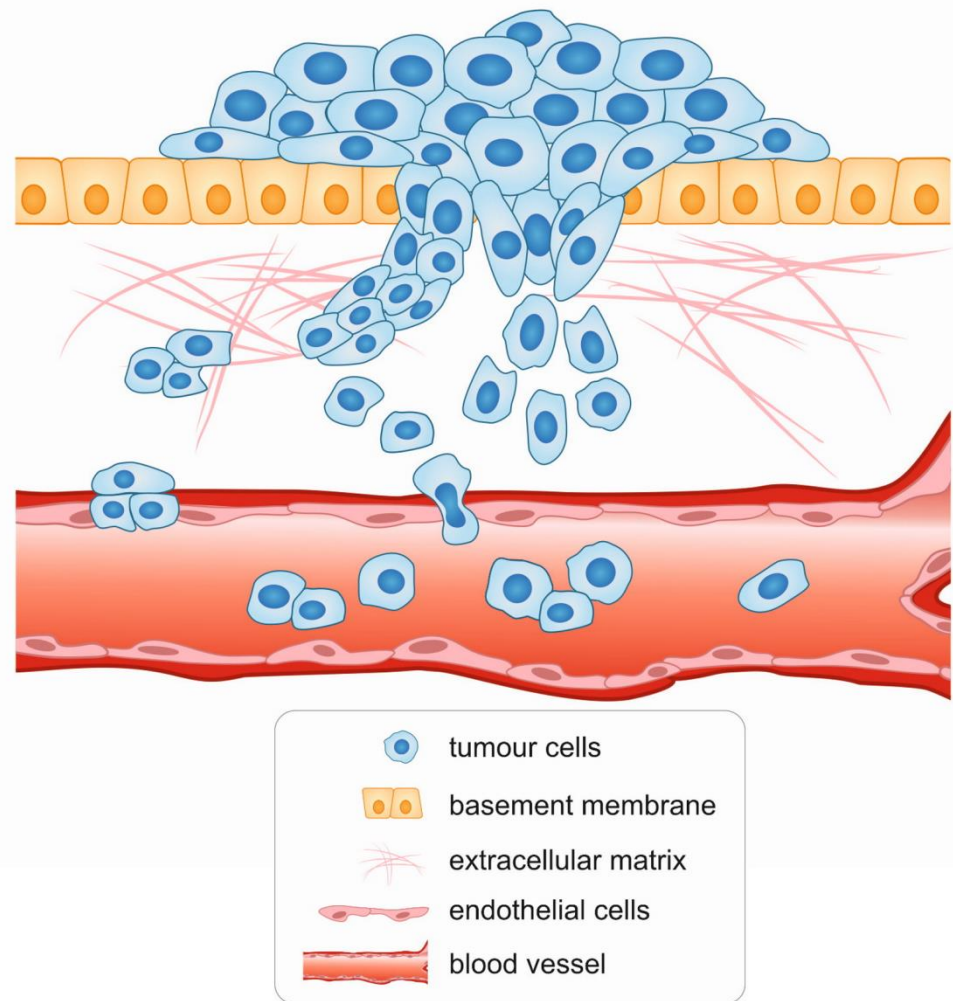
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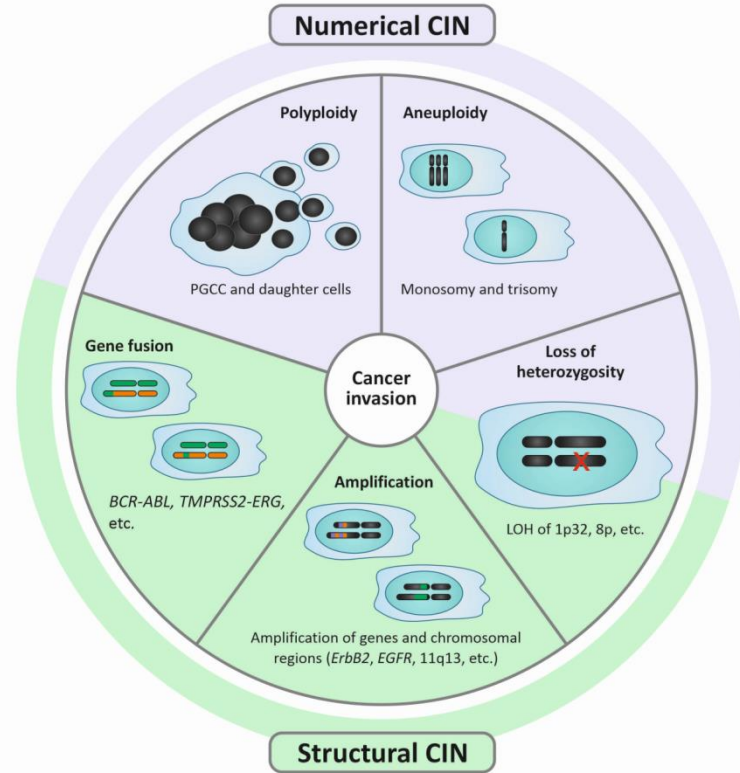


1248

1249 **Fig. 1. The model of cancer cell invasion**

1250 Cancer invasion is the first step of the metastatic cascade. Tumour cells penetrate the
 1251 basement membrane and invade surrounding tissues using two modes of movement – individual
 1252 and collective invasion. Invading tumour cells reach the blood vessel, enter the blood flow and
 1253 disseminate, eventually giving rise to secondary tumours.

1254



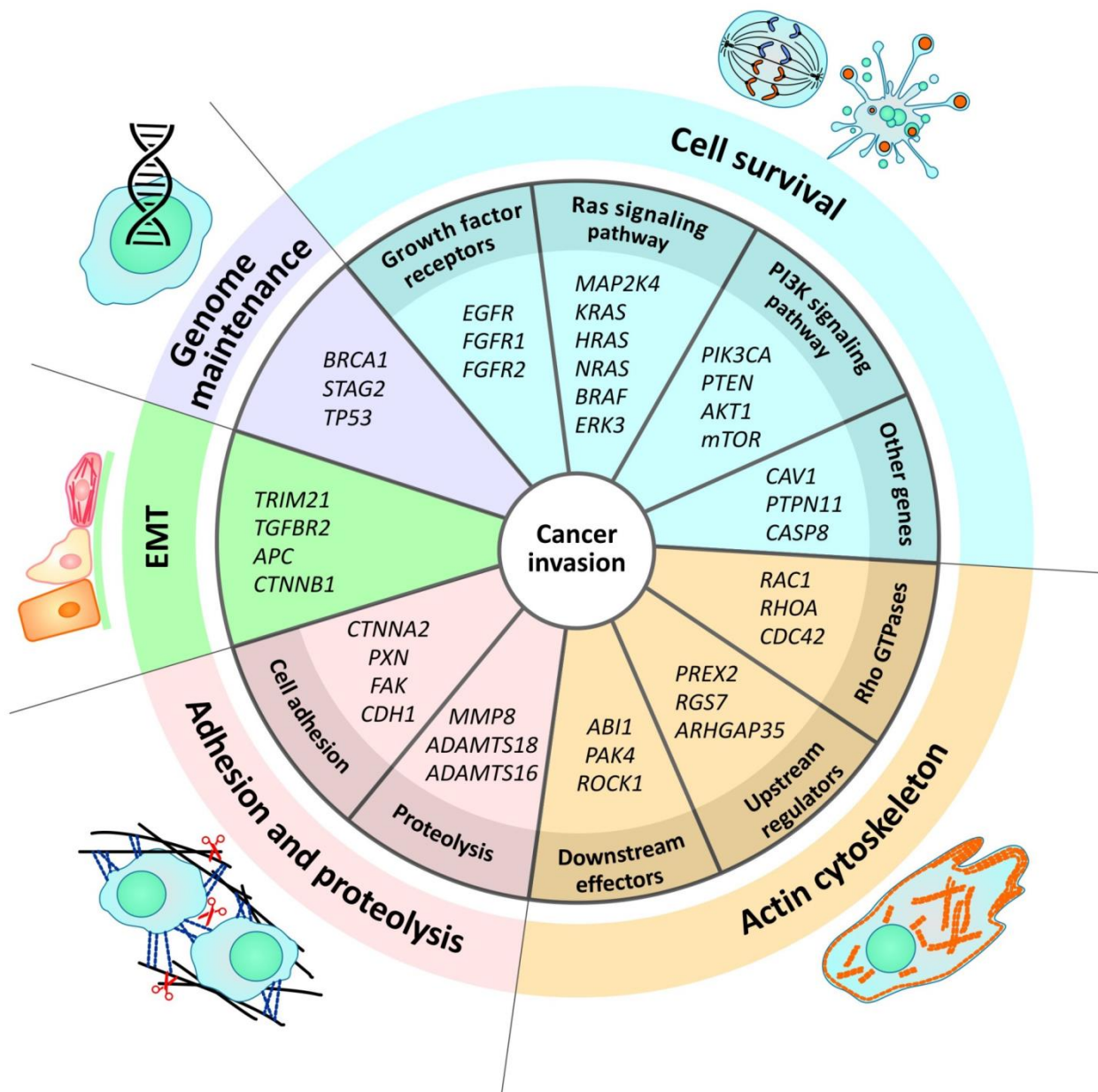
1255

1256 **Fig. 2. Chromosomal instability and cancer invasion**

1257 Chromosomal instability (CIN) is one of the cancer hallmarks and plays an important role
 1258 in tumour cell migration and invasion. CIN can be represented by gain or loss of whole
 1259 chromosomes (numerical CIN) and chromosomal rearrangements (structural CIN). Loss of
 1260 heterozygosity (LOH) that can be attributed to numerical and structural CIN simultaneously
 1261 depending on the type of genomic changes resulting in the allele loss affects the invasive
 1262 potential of tumour cells. Polyploidy defined as the presence of additional sets of chromosomes
 1263 drastically changes the genetic landscape of tumour cells, endowing them with high invasive
 1264 potential. Polyploid giant cancer cells (PGCCs) are found in various cancers and show extreme
 1265 tumourigenic, invasive, and metastatic potential. Aneuploidy when chromosomes can be lost
 1266 (monosomy) or gained (trisomy) can have different effects on tumour cell invasion: from
 1267 attenuation of migratory behaviour to its enhancement. Different gene fusions arising from
 1268 various chromosomal rearrangements affect tumour cell motility through diverse signalling
 1269 pathways and mechanisms. Amplification defined as a copy number increase of a certain region

1270 of the genome leads to enhanced gene expression and, if gene positively regulates cellular
 1271 motility, can accelerate cancer invasion.

1272



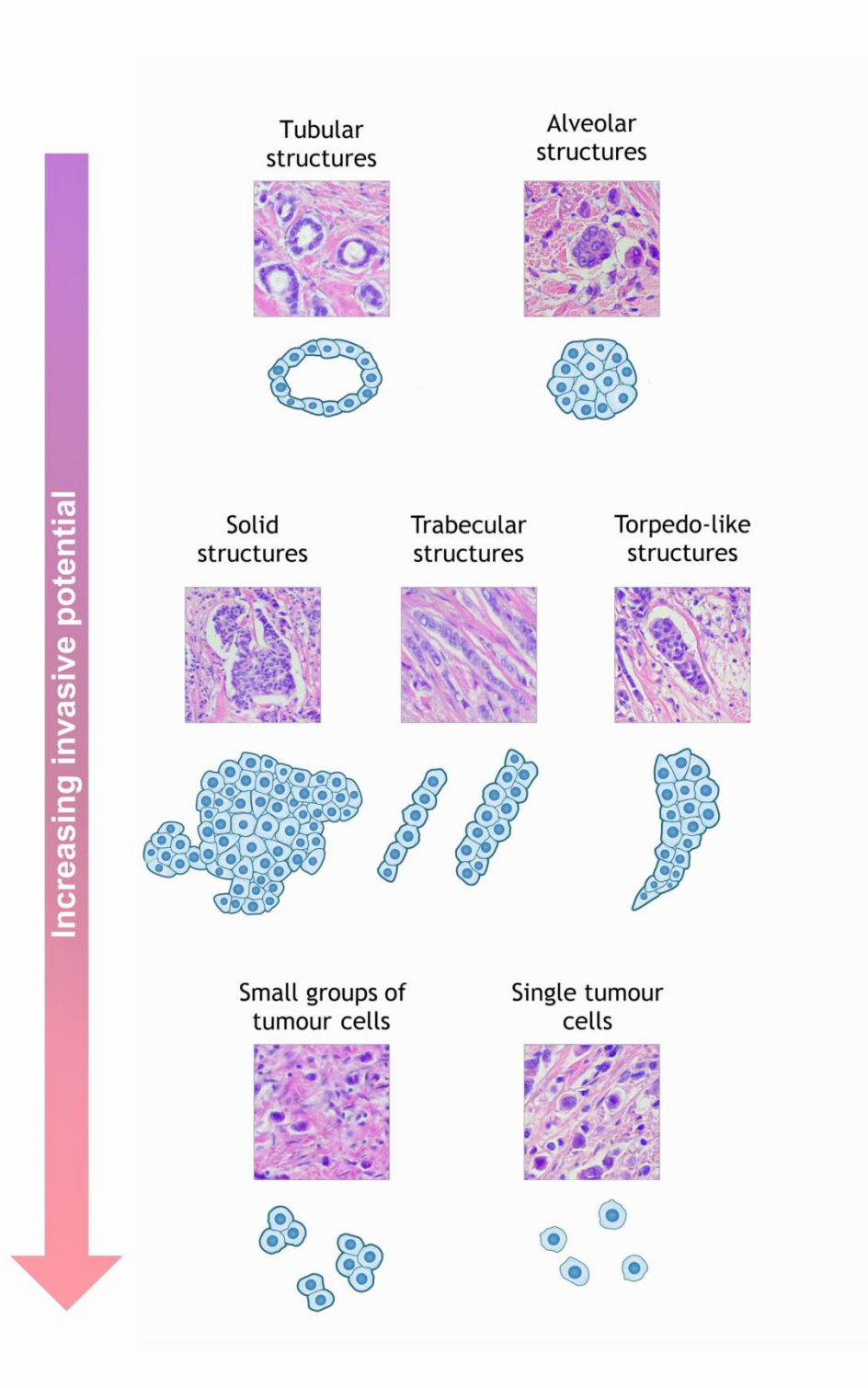
1273

1274 **Fig. 3. Gene alterations and cancer invasion**

1275 Various gene mutations can affect tumour cell migration and invasion. Genes responsible
 1276 for genome maintenance are frequently mutated in cancers; however, only a few of them can
 1277 influence tumour cell motility, the main player here being *TP53* and its diverse mutant forms.
 1278 Alterations in genes that play a role in cell survival affect a variety of cellular processes and
 1279 signalling pathways underlying cell migration. Mutations in genes encoding regulators of the

1280 actin cytoskeleton, adhesion, proteolysis, and EMT directly influence the ability of tumour cells
1281 to migrate and invade.

1282



1283

1284 **Fig. 4. Intratumoural morphological heterogeneity of breast cancer as a model for**
1285 **studying the mechanisms of tumour cell invasion**

1286 Intratumoural morphological heterogeneity of invasive carcinoma of no special type, the
 1287 common histological type of breast cancer, is represented by various types of architectural
 1288 arrangements of tumour cells that significantly differ in the transcriptomic profile namely in the
 1289 expression of genes involved in EMT and enrichment of cancer invasion signaling pathways.
 1290 Tubular and alveolar structures are similar in epithelial and mesenchymal gene expression
 1291 patterns. Solid structures demonstrate an increase in mesenchymal markers but retain epithelial
 1292 features. Trabecular structures display a pronounced mesenchymal phenotype and a dramatic
 1293 decrease in epithelial traits. Small groups of tumour cells and single tumour cells show a strong
 1294 mesenchymal phenotype and the significant enrichment of cancer invasion signalling pathways.
 1295 Torpedo-like structures have been recently identified to be associated with breast cancer
 1296 metastasis through the activity of kinesin-14 (KIF14), mitochondria-eating protein (Mieap), and
 1297 ezrin (EZR) that are known regulators of tumour cell motility and invasion. However, the EMT
 1298 degree of torpedo-like structures remains to be elucidated. Based on these data, it can be
 1299 hypothesized that tubular and alveolar structures are less invasive whereas solid, trabecular, and
 1300 torpedo-like structures, as well as small groups of tumour cells and single tumour cells, are
 1301 highly invasive. In addition, considering the architectural features, solid, trabecular, and torpedo-
 1302 like structures, as well as small groups of tumour cells, can be attributed to collective cancer cell
 1303 invasion whereas single tumour cells – to individual cancer cell invasion.

1304

1305 **Table 1.** Genetic alterations associated with migration and invasion of different cancer
 1306 cells

Cancer	Genetic alterations
Breast cancer	Chromosomal instability: polyploidy, <i>ESR1-YAPI</i> and <i>ESR1-PCDH11X</i> fusions, <i>ERBB2</i> amplification, LOH of 8p22 (<i>DLCL1</i>), and LOH of 8p Gene alterations: <i>BRCA1</i> , <i>TP53</i> , <i>NRAS</i> , <i>PIK3CA</i> , <i>RBI</i> , <i>CAVI</i> ,

	<i>PTPN11, ARHGAP35, FAK, CDH1, ADAM12, ADPGK, PCGF6, PKP2, NUP93, SLC22A5, and TRIM21</i>
Colorectal cancer	Chromosomal instability: polyploidy, trisomy of chromosomes 5, 7, 13, and 18 Gene alterations: <i>TP53, KRAS, BRAF, PIK3CA, APC, and SMAD4</i>
Prostate cancer	Chromosomal instability: <i>TMPRSS2-ERG</i> fusion and LOH of 8p22 (<i>DLC1</i>) Gene alterations: <i>PTEN, RB1, AB11, PAK4, and ITGA7</i>
Non-small cell lung cancer	Chromosomal instability: <i>FGFR1</i> and <i>SNHG17</i> amplifications, and LOH of 8p22 (<i>DLC1</i>) and 1p32 (<i>TGFBR3</i>) Gene alterations: <i>TP53, EGFR, FGFR1, ERK3, MAP2K4, AKT1, PXN, EPHB6, and ND6</i>
Melanoma	Chromosomal instability: miR-182 amplification Gene alterations: <i>TP53, BRAF, RAC1, RGS7, MMP8, and GRM3</i>
Head and neck squamous cell carcinoma	Chromosomal instability: 11q13 amplification Gene alterations: <i>PIK3CA</i> and <i>CASP8</i>
Oral squamous cell carcinoma	Chromosomal instability: 11q22.1-q22.2 amplification Gene alterations: <i>TGFBR2</i> and <i>NOTCH1</i>

1307

1308 **Box 1. A brief overview of the processes responsible for CIN**

1309 CIN, one of the forms of genomic instability in tumours, is characterized by an increase in
1310 the rate of loss or gain of whole chromosomes or their fragments during cell division. CIN has a
1311 severe and complex impact on the genetic landscape of the tumour by affecting various
1312 oncogenes, tumour suppressor genes, and DNA repair genes that drive cancer growth and
1313 progression. CIN promotes intratumoural heterogeneity and clonal evolution, giving cancer cells

1314 an advantage under selective pressure.⁸

1315 Different mitotic events underlie CIN. Among them are cohesion defects, dysfunction in
1316 spindle assembly checkpoint, centrosome amplification, and cytokinesis failure. Defects in DNA
1317 replication and repair, such as telomere dysfunction and replication stress, are also responsible
1318 for CIN. All these changes lead to chromosome missegregation during mitosis and pave the way
1319 to polyploidy, aneuploidy, and diverse chromosomal rearrangements.^{212,213}

1320 The role of CIN in cancer growth and progression remains debatable. Some researchers
1321 consider CIN to be an early event in cancer, and some believe that CIN is simply a side effect of
1322 tumour growth.⁸ In any event, CIN is significantly associated with drug resistance and cancer
1323 progression.^{8,10}

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