Metabolic reprogramming and epithelial-to-mesenchymal transition in cancer

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Abstract

Several lines of evidence indicate that during transformation epithelial cancer cells can acquire mesenchymal features via a process called epithelial-to-mesenchymal transition (EMT). This process endows cancer cells with increased invasive and migratory capacity, enabling tumour dissemination and metastasis. EMT is associated with a complex metabolic reprogramming, orchestrated by EMT transcription factors, which support the energy requirements of increased motility and growth in harsh environmental conditions. The discovery that mutations in metabolic genes such as *FH*, *SDH* and *IDH* activate EMT provided further evidence that EMT and metabolism are intertwined. In this review, we discuss the role of EMT in cancer and the underpinning metabolic reprogramming. We also put forward the hypothesis that, by altering chromatin structure and function, metabolic pathways engaged by EMT are necessary for its full activation.

Background

In the last decades, cancer research uncovered the many enabling features of tumours cells [1]. Among these, activation of epithelial-to-mesenchymal transition (EMT), a process where epithelial cancer cells acquire mesenchymal features, is emerging as key determinant of cancer cell invasion and metastasis [2-4]. To metastasise, cancer cells acquire the ability to erode the extracellular matrix, the motility to extravasate into the blood stream, and the plasticity to grow in a different tissue. In all these phases, nutrient supply can be limited and cancer cells experience varying degree of stress [5]. Accordingly, metastatic cells fine-tune their metabolism to adapt to the ever-changing environment [6, 7]. In line with this observation, part of the genetic reprogramming orchestrated by EMT affects the expression of metabolic genes, regulating glucose, lipids, glutamine, and nucleotide metabolism. Yet, to what extent EMT rewires the metabolic network is still unclear. The recent discovery that oncogenic mutations of metabolic enzymes such as fumarate hydratase (FH), succinate

34 dehydrogenase (SDH) and isocitrate dehydrogenase (IDH) drive EMT [8-10] indicates that the
35 connection between EMT and metabolism is deeper than anticipated. Indeed, these works revealed
36 that components of the metabolic network can directly affect chromatin structure and function,
37 impinging on signalling cascades required for the full activation of EMT [11, 12]. In this review, we
38 describe the role of EMT in tumorigenesis, how EMT affects metabolism, and how, in turn,
39 dysregulation of metabolic genes affect the execution of EMT.

40 The epithelial-to-mesenchymal transition in cancer

41 In tissues, epithelial cells are organised in compact layers anchored to the basal lamina. During
42 transformation, some of these cells lose their epithelial features and acquire a mesenchymal
43 phenotype through a process defined as epithelial-to-mesenchymal-transition (EMT). This process is
44 characterised by profound transcriptional [13] and epigenetic changes [14, 15] that lead to the loss of
45 cell-to-cell junctions and the acquisition of a motile and migratory phenotype, enabling the invasion
46 of the basal lamina, which eventually may lead to metastasis. At the molecular level, EMT is dictated
47 by a network of transcription factors (EMT-TFs) that directly or indirectly represses one of the key
48 epithelial markers, E-Cadherin [13, 16]. These EMT-TFs belongs to various family of chromatin
49 interacting family of proteins, including Snail (Snai1 and Snai2), bHLH (Twist1 and Twist2), and zinc
50 finger and E-box binding (Zeb1 and Zeb2). Cross-activation of EMT by other oncogenic stimuli and the
51 identification of non-canonical EMT-TFs such as Kruppel-like-factor (KLF8), the homebox proteins
52 goosecoid (GSC) or fork-head protein (FOXC2), contributes to the great complexity of EMT regulation
53 [13, 16]. Moreover, recent evidence has shown that microRNAs are also potent regulators of EMT,
54 affecting the expression of multiple targets of this cascade [17].

55 The role of the EMT-TFs in invasion and metastasis has been extensively investigated [16]. *In*
56 *vivo* experiments using a spontaneous squamous cell carcinoma mouse model showed that the
57 expression of the EMT-TF *Twist1* is sufficient to trigger EMT and the subsequent dissemination of
58 cancer cells into the blood stream. Interestingly, the colonisation of target tissues by these metastatic
59 cells is driven by a mesenchymal-to-epithelial transition (MET) and requires suppression of *Twist1* [18].
60 Other works identified a primary role of the EMT in breast cancer progression. For instance, *Twist1*
61 controls the ability of aggressive breast 4T1 cells to migrate *in vitro* and to metastasise to the lung *in*
62 *vivo* [19]. The role of Twist1 in early dissemination and metastasis was also corroborated in human
63 epidermal growth factor receptor 2 (Her2)-positive mammary cancer cells. It was shown that in early
64 lesions in mouse breast, a subpopulation of cells that express high levels of *Twist1*, low levels of E-
65 cadherin, and markers of Wnt signalling activation, invade the adjacent tissue and lead to early
66 dissemination and subsequent mestastasis [20]. Moreover, in mouse skin squamous cell carcinoma,
67 *Twist1* is required in both early and late stages of tumour progression in a gene dosage- dependent

68 manner [21]. Other EMT-TF are directly involved in breast cancer metastasis. For instance, the
69 expression of *Snai1* in a mouse model of breast cancer activates the dissemination of cancer cells and
70 its deletion dramatically impairs the formation of metastasis [22]. The impact of *SNAI1* activation in
71 the malignancy of breast tumours has been further confirmed by the discovery that the discoidin
72 domain receptor 2 (DDR2), a protein expressed in ductal breast carcinomas, drives invasion *in vitro*
73 and metastasis *in vivo* through the nuclear stabilisation of *Snai1*, via phosphorylation mediated by
74 extracellular related kinase 2 (ERK2) [23]. Even though a series of convincing works established the
75 involvement of EMT in metastasis formation, its real importance in tumour evolution is still
76 questioned. For instance, two groups recently showed that the EMT is dispensable for metastasis in a
77 model of pancreatic [24] and breastcancer [25]. These results suggest that the role of EMT in cancer
78 progression is likely tissue-specific and that it might be implicated in other features of cancer. Indeed,
79 it has recently emerged that EMT, via the expression of EMT-TFs, enables stemness in cancer cells [2,
80 16]. For instance, an orchestrated signal mediated by *SNAI2* and *SOX9* induces a stem state and
81 promotes tumorigenesis in mammary luminal cells [26], while the ectopic expression of *TWIST1* or
82 *SNAI1* results in the expression of stem markers in human immortalised mammary cells [27].
83 Moreover, ZEB1-mediated suppression of miR200 favours the expression of polycomb repressor
84 protein Bmi1 [28, 29] and Suz12 [30], two regulators of self-renewal and stemness in breast cells.
85 Further work showed that the acquisition of stem-like properties through EMT activation is involved,
86 at least in part, in both chemoresistance [31, 32] and tumour dormancy [31, 33-35]. These two
87 prominent features of cancer therapy may be interlinked. Seminal work using an elegant *in vivo* model
88 to trace EMT lineage during metastasis showed that EMT-positive cells are responsible for recurrence
89 of lung metastasis after chemotherapy with cyclophosphamide, suggesting that chemoresistance,
90 EMT and dormancy may be part of the same pathway [25].

91 EMT activation induces a metabolic rewiring

92 Recent findings indicate that mesenchymal cancer cells have different metabolic needs compared
93 their epithelial counterparts, to satisfy the metabolic demands of increased motility and invasion. Yet,
94 how EMT regulates metabolism is still poorly understood. In the effort to corroborate this link, Shaul
95 and colleagues analysed the expression of metabolic genes in high-grade carcinomas expressing
96 mesenchymal markers using publically available data from almost 1000 cancer cell lines. They found
97 that these mesenchymal cells exhibit high expression levels of 44 metabolic genes. These genes were
98 found upregulated also upon induction of EMT by expression of *Twist1* in human mammary epithelial
99 cells. Among these enzymes, Dihydropyrimidine dehydrogenase (DPYD), an enzyme involved in
100 pyrimidine catabolism, was required for EMT, both *in vitro* and *in vivo* [36] (Figure 1). Importantly,
101 exogenous dihydropyrimidines are sufficient to rescue EMT after silencing of DPYD, suggesting that

102 these metabolites are a limiting factor during the EMT. However, the how they regulate EMT is
103 currently unknown.

104 Overall, these results suggested that metabolic rewiring is required to complete the
105 reprogramming orchestrated by EMT. In further support of these findings, it was found that *SNAI1*
106 expression represses the glycolytic enzyme fructose-1,6-bisphosphatase 1 (FBP1), favouring glucose
107 uptake and the diversion of glycolytic carbons towards biosynthetic pathways, including the pentose
108 phosphate shunt (Figure 1). Interestingly, FBP1 loss impairs respiration and the activity of respiratory
109 chain complex I [37]. Activation of glycolysis by EMT was also observed in breast and prostate cancer
110 cells, where it is required for both cytoskeleton remodelling and increasing cell traction [38]. Glycolysis
111 is targeted by EMT also in non-small cell lung cancer cells (NSCLC), where ZEB1 activate the expression
112 of glucose transporter 3 (*GLUT3)* [39]. However, the metabolic reprogramming upon EMT in NSCLC is
113 controversial. For instance, the treatment of NSCLC with TGF-β induces a shift from glycolysis to
114 OXPHOS and leads to an overall increase in amino acids, in particular in glutamate, via a higher flux of
115 carbons through the TCA cycle. Mechanistically, this shift from glycolysis to OXPHOS is achieved by a
116 selective repression of pyruvate dehydrogenase kinase 4 during EMT [40]. Finally, EMT induction by
117 TGF-β in colon cancer cells elicits the nuclear translocation of pyruvate kinase M2 (PKM2) and the
118 silencing of PKM2 prevents EMT triggering by TGF-β in these cells [41] (Figure 1).

119 Other metabolic pathways are targeted during EMT, including lipid metabolism (Figure 1). For
120 example, EMT activation by either TNFα or TGF-β favours the accumulation of unsaturated
121 triacylglycerides in DU145 prostate cancer cells [42]. Furthermore, the activation of EMT by
122 overexpression of *SNAI1* suppresses transcriptional regulators of the lipogenesis carbohydrate-
123 responsive element binding protein (ChREBP) leading to the silencing of both fatty acid synthase
124 (FASN) and acetyl-CoA carboxylase (ACC) [43]. Finally, another pathway required during EMT is
125 glutaminolysis (Figure 1): lung cancer cells that undergo an EMT become increasingly sensitive to
126 Glutaminase-1 (GLS1) inhibitors [44].

127 As discussed above, EMT activation is involved in both chemoresistance and tumour dormancy.
128 Even though the role of metabolism in these processes is largely unknown, recent works suggest that
129 metabolic rewiring can be important in both chemoresistance and tumour dormancy. For instance,
130 EMT-positive breast cells that are responsible for recurrent lung metastasis after chemotherapy
131 increased the expression of metabolic enzymes such as drug transporters, aldehyde dehydrogenase
132 (ALDHs), cytochrome P450s, and enzymes of glutathione metabolism [25] (Figure 1). Likely, these
133 metabolic changes protect the cells from oxidative stress experienced during therapy. Furthermore,
134 deletion of *Twist1* or *Snai1* in chemoresistant pancreatic cancer cells increase the expression of a
135 nucleosides transporter, which leads to increase uptake of the anticancer drug gemcitabine [24]. The

136 link between EMT, metabolic alterations, and tumour dormancy remains mainly indirect. It is widely
137 known that during tumour dormancy, cancer cells undergo proliferative arrest and enter quiescence
138 [34]. Therefore, it not surprising that this change in proliferation rate is accompanied by a metabolic
139 rewiring. For instance, pancreatic ductal cancer cells surviving after oncogene ablation acquire stem-
140 like traits and are dependent on oxidative phosphorylation for survival [45]. In addition, quiescent
141 leukaemia stem cells (LSC) rely on mitochondrial metabolism: targeting the oxidative phosphorylation
142 through BCL-2 inhibition is sufficient to eradicate LSC population [46]. However, the impact of EMT-
143 TFs in regulating these metabolic alterations during dormancy is largely unknown and it might be
144 related to the dynamic shift between EMT and MET that occurs on tumour circulating cells [47].

145 Overall, these results suggest that metabolic reprogramming is instrumental to the phenotypic shift
146 observed during the EMT. Whether these metabolic changes are simply required to fulfil the energy
147 requirements of more aggressive cells or to support some of the signalling cascades involved in this
148 process is still unknown.

149 Metabolic reprogramming activates the epithelial- to-mesenchymal transition

150 Recent evidence suggests that the link between EMT and metabolism is mutual and, in some
151 circumstances, alterations of metabolism can drive EMT. The next part of the review describes how
152 the dysregulation of metabolic pathways is associated with EMT induction. These findings are
153 summarised in Figure 2.

154 *Glycolysis*

155 Aerobic glycolysis is the most distinctive metabolic alteration of cancer cells [1, 48] but the role of
156 glycolytic enzymes in the induction of EMT has emerged only in the last years. Phosphoglucose
157 isomerase (PGI) is a glycolytic enzyme that converts glucose-6P to fructose 6-P. This enzyme was found
158 to be secreted by cancer cells and to act as cytokine, taking the name of autocrine motility factor
159 (AMF). Overexpression of PGI/AMF causes a NF-kB-dependent stabilisation of ZEB1 and ZEB2 in breast
160 cancer cells [49] and ectopic expression in normal epithelial breast MCF10A triggers EMT [50].
161 Importantly, suppression of PGI/AMF leads to reverse MET in lung fibrosarcoma [51] and endometrial
162 cancer cells [52]. As described above, the expression of the glycolytic enzyme fructose-1,6-
163 biphosphatase (FBP1) blocks the induction of EMT mediated by SNAI1 in luminal breast cells. The
164 silencing of FBP1 favours EMT also in gastric cells *in vitro* [53]. Other glycolytic enzymes are involved
165 in EMT induction. For instance, the silencing of Aldolase A (ALDOA), an enzyme that converts fructose-
166 1,6-bisphosphate to glyceraldehydes-3-phosphate and hydroxy-acetone, impairs lung squamous
167 carcinoma cell motility and tumorigenesis and this phenomenon is associated with repression of
168 mesenchymal markers [54]. Furthermore, silencing of glyceraldehyde-3-phosphate dehydrogenase
169 (GAPDH) inhibits EMT by repressing *SNAI1* in colon cancer [55]. Finally, overexpression of lactate

170 dehydrogenase (LDH), the enzyme that converts pyruvate to lactate, leads to increased migration and
171 invasion in bladder cancer cells [56].

172 *Mitochondrial metabolism*

173 Mitochondrial dysfunction is a key feature of cancer and has been frequently associated with
174 increased aggressiveness and metastatic potential [57, 58]. Yet, the mechanistic link between
175 mitochondrial dysfunction and EMT have only recently been investigated. In 2014 it was shown that
176 mitochondrial dysfunction induced by depletion of mitochondrial DNA in breast cells leads to
177 profound morphological and molecular changes that resembles EMT, including increased expression
178 of EMT-TFs, metalloproteases and suppression of E-cadherin, triggered by a Calcineurin A (CaN)-
179 dependent mechanism [59]. In support of this finding, we recently found that the downregulation of
180 mitochondrial genes is a common feature of highly aggressive cancers, and that it significantly
181 correlates with the activation of EMT across 21 different types of cancer [60]. More recently, we and
182 others have demonstrated that EMT is a key signature of tumours harbouring mutations in the
183 Tricarboxylic Acid (TCA) cycle enzymes *FH*, *SDH* and *IDH* [8-10].

184 Fumarate hydratase is the enzyme that converts fumarate to malate. Mutations of this enzyme lead
185 to Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC) [61] and other tumour types, including
186 paragangliomas and pheochromocytomas [62, 63], whilst FH deletions have been found in
187 neuroblastoma [64]. FH-mutant renal tumours are highly aggressive and metastasise even when small
188 [65]. However, the mechanisms underpinning this aggressiveness are still under investigation. We
189 recently demonstrated that FH-deficient cells exhibit a striking mesenchymal phenotype, linked with
190 the expression of an EMT signature [8]. The link between FH and EMT was also observed in
191 nasopharyngeal carcinoma, where FH is transcriptionally repressed by the lymphoid-specific helicase
192 (LSH) [66]. Mechanistically, we found that fumarate, which accumulates in FH-deficient cells and
193 tumours, is responsible for the induction of EMT by inhibiting the TET-dependent demethylation of
194 the anti-metastatic microRNA miR200 [8], known inhibitors of both SNAI2 [67] and ZEB1 [68] (Figure
195 3).

196 Another TCA cycle enzyme implicated in EMT is Succinate dehydrogenase (SDH), a component of the
197 respiratory chain that converts succinate to fumarate. *SDH* mutations have been described in
198 pheochromocytomas and paragangliomas [69-72], sporadic renal cancer [73] and gastrointestinal
199 stromal tumours [74, 75]. A recent study revealed that human metastatic pheochromocytomas and
200 paragangliomas harbouring *SDHB* mutations are invasive and exhibit activation of EMT–TFs such as
201 SNAI1 and SNAI2, suggesting the induction of EMT in these tumours [10] . Consistently, it was shown
202 that loss of SDHB in chromaffin cells induces these EMT-TFs and leads to the epigenetic silencing of
203 keratin-19 [76, 77]. Importantly, the migratory phenotype of these cells is reversed by the use of a

204 DNA methylation inhibitor, decitabine. The link between *SDHB* deficiency and EMT was also shown in
205 colorectal cancer, where the silencing of *SDHB* promotes cell migration and invasion in a TGF-β/SNAI1-
206 mediated-process [78], and also in ovarian cancer [79]. Finally, loss of the assembly factor SDH5 [80],
207 induces EMT in lung cancer cells and metastasis *in-vivo* through activation of a glycogen-synthase
208 kinase (GSK-3β)-β-catenin axis [81]. Although these studies did not focus on the accumulation of
209 succinate as a mediator of EMT, we recently found that succinate, similarly to fumarate, can induce
210 the epigenetic suppression of miR200 and subsequent EMT induction in Sdhb-deficient epithelial
211 kidney cells [8] (Figure 3).

212 Other TCA cycle enzymes recently appeared in the spotlight of cancer biology and EMT are Isocitrate
213 Dehydrogenases (IDHs), enzymes involved in the oxidative decarboxylation of isocitrate to alpha-
214 ketoglutarate (aKG). Three isoforms of IDH have been identified: cytosolic IDH1 and mitochondrial
215 IDH2 are $NADP^+$-dependent enzymes, while mitochondrial IDH3 is a NAD^+-dependent protein.
216 Heterozygous mutations in either *IDH1* or *IDH2* have been found in gliomas and leukaemia [82-84].
217 *IDH1* and *IDH2* mutations are neomorphic and lead to the production of 2-hydroxyglutarate (2HG),
218 which was shown to induce EMT. Similar to what was described for FH and SDH deficient cells, EMT in
219 IDH-mutant cells is driven by alterations of the *miR200-Zeb1* axis (Figure 3). This phenomenon was
220 observed in breast tumours [9], and in colorectal cancer cells [9, 85].

221 Finally, another TCA cycle enzyme associated with EMT is citrate synthase (CS), the enzyme that
222 catalyses the first committed step of the TCA cycle. Silencing of CS induces morphological and
223 molecular changes in human cervical carcinoma cells that resemble EMT, and promotes metastasis *in*
224 *vivo*. The molecular mechanisms responsible for this phenotype are not clear, but it is possible that
225 the mitochondrial dysfunction observed in these cells is involved [86]. However, more recent
226 experiments indicate that CS is upregulated in other tumour types such as ovarian cancers and that
227 its silencing impairs both motility and invasion of tumour cells *in vitro* [87]. Therefore, the role of CS
228 in tumour progression is still unclear and it might be tissue-dependent.

229 *Lipid metabolism*

230 Several recent reports support the connection between lipid metabolism and EMT. For instance, the
231 overexpression of acetyl-CoA synthetase (ACSL1 and ACSL4) and steroyl-CoA desaturase (SCD) can
232 activate EMT in colorectal cancer, leading to increased migration, invasion and colony formation *in*
233 *vitro*. Importantly, the expression of these three enzymes is associated with poor prognosis in stage II
234 colorectal cancer patients [88]. In addition, elevated fatty acid uptake via CD36 activates a Wnt-
235 dependent EMT in hepatocellular carcinoma (HCC) [89]. Of note, in human oral cancer cells CD36-
236 positive cells are responsible for cancer initiation and metastasis *in vivo.* However, in the latter model
237 the EMT is not involved in the formation of metastasis [90]. Other enzymes of lipid metabolism have

238 been identified as EMT regulators. For instance, silencing of ATP citrate lyase (ACL) reverses EMT in
239 lung cancer and impairs stemness in both lung and breast cells by SNAI1 repression [91]. Moreover,
240 silencing of acetyl-CoA carboxylase 2 (ACC2) reverted the EMT transition triggered by glucose stress,
241 triglyceride deposit and malonyl-CoA accumulation in kidneys [92]. Interestingly, treatment of cancer
242 cells with fatty acids such as arachidonic or linoleic acid elicits an EMT that is downstream of the
243 oncogenic cascades mediated by SRC, NF-kB and FAK [93, 94].

244 *Glutaminolysis*

245 Most cancer cells depend on glutamine utilisation [48], and the role of glutaminolysis in EMT has been
246 recently investigated. The inhibition of glutaminolysis by targeting GLS1 impairs *in vivo* metastasis
247 through repression of *SNAI1* [95]. On the contrary, the expression of GLS2, the mitochondrial isoform
248 of glutaminase, inversely correlates with stage, tumour size, and prognosis in HCC. However, this
249 phenomenon is independent of GLS2 glutaminase activity and involves the GLS2-mediated
250 stabilisation of the EMT-related microRNA miR-34a *via* the Dicer complex [96]. These results suggest
251 that the effects of glutamine catabolism on EMT might be context-dependent and more work is
252 necessary to elucidate the importance of glutaminolysis in this process.

253 Conclusions and future perspective

254 EMT is a fundamental biological process involved in development, fibrosis, and wound healing [4].
255 Recent evidence indicates that this process is also involved in tumour initiation and metastasis. EMT
256 elicits a complex phenotypic switch that endows cancer cells with ability to survive during invasion,
257 dissemination, and metastasis. This flexibility is achieved at least in part by the rewiring of the
258 metabolic network. As discussed above, EMT, via EMT-TFs, orchestrates profound metabolic changes
259 that allow the cell to sustain the energy needs of a cancer cell in an ever-changing tumour micro
260 environment. Yet, the role of metabolism in EMT seems to go beyond these simple enabling features.
261 Indeed, the observation that dysregulation of cellular metabolism, in some circumstances, drives EMT
262 indicates that parts of the metabolic network could act as a core component of the signalling cascade
263 elicited by the EMT (Figure 4). The data discussed in this review corroborate this hypothesis and
264 indicate that specific metabolic alterations could lead to chromatin changes that are required for the
265 activity of EMT-TFs. Several questions arise. For instance, it is still unclear why different sources of
266 mitochondrial dysfunction converge on EMT. In an interesting parallel, EMT induction is associated
267 with bypass of oncogene –induced senescence [97]. Given that senescence is a common outcome of
268 metabolic stress [98] it is possible that induction of EMT could provide cells with the sufficient
269 plasticity to survive and proliferate in the presence of metabolic defects or under nutrient stress. In
270 this scenario, metastasis could be seen as a strategy to explore novel, and more favourable, metabolic
271 niches, and increased motility the means to this goal. Another outstanding question in the field is to

272 what extent the EMT observed in metabolically-impaired cells contributes to tumorigenesis. The fact
273 that EMT is the most enriched gene signature in FH and SDH-deficient cells seems to support a driving
274 role of EMT in these tumours. It would be important to validate this hypothesis by assessing
275 tumorigenesis in FH- or SDH-deficient models where EMT-TFs are ablated. Finally, the fact that EMT
276 shows unexpected metabolic facets offers interesting therapeutical perspectives (Fig.4). Indeed, EMT
277 could be potentially reverted by targeting specific metabolic enzymes, or targeting the metabolism-
278 dependent epigenetic reprogramming, eventually limiting cancer metastasis. Consistently, inhibitors
279 of mutant IDH were shown to revert glioma cells to a more differentiated state [99], and the DNA
280 methylation inhibitor, decitabine, impairs the invasive phenotype of SDH-deficient cells [77]. Along
281 this strategy, a recent screening was designed to identify small molecules that could revert the
282 mesenchymal phenotype of cancer cells activating E-cadherin transcription. Interestingly, it was found
283 that protein kinase A (PKA) activation by increasing cyclic AMP (cAMP) levels, is sufficient to trigger a
284 mesenchymal-to-epithelial transition (MET) in aggressive breast cancer cells, through activation of the
285 histone demethylases PHF2. cAMP is a key second messenger and its levels are tightly controlled by
286 the energy state of cells [100]. Therefore, it is tempting to speculate that metabolic alterations,
287 through regulation of cAMP levels, are necessary for full EMT activation and that altering metabolism
288 could be a tempting strategy to modify cell phenotype and, more importantly, aggressive features of
289 cancer.

290 Overall, in this review we provided compelling evidence that EMT and metabolism are intertwined.
291 Understanding the underpinning molecular determinants of this relation is revealing novel insights
292 into how tumours are formed and disseminate, and will potentially provide novel targets for targeting
293 metastasis, the major killer in cancer.

294

295 **Competing interests**

296 The authors declare no competing interests.

297 **Authors' contribution**

298 MS and CF jointly wrote the manuscript.

299 **Authors' information**

300 MS is a Research Associate in the laboratory of CF. CF is a group leader at the MRC Cancer Unit,
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Figure legends

Fig.1 EMT controls metabolic reprogramming

EMT transcription factors (EMT-TFs) control the expression of metabolic genes of different pathways such as glycolysis, lipid metabolism, and mitochondrial metabolism, and glutaminolysis. Specifically, EMT-TFs suppress the expression of fructose-1,6-bisphosphatase 1 (FBP1), fatty acid synthase (FASN), acetyl-coA carboxylase (ACC), nucleoside transporter, and pyruvate dehydrogenase kinase 4 (PDK4), whilst enhance the expression of dihydropyrimidine dehydrogenase (DPYD), glutaminase 1 (GLS1), enzymes of glutathione metabolism, cytochrome P450, aldehyde dehydrogenases, and glucose transporter 3 (GLUT3). Red dashed arrows indicate the metabolic nodes regulated by EMT-TFs. TCA=tricarboxylic acid cycle.

Fig.2 Metabolic genes control EMT.

Aberrant expression of metabolic enzymes of glycolysis (orange), lipid metabolism (purple), glutaminolysis (blue), mitochondrial metabolism (green), leads to EMT. Red dashed arrows indicate the link between specific metabolic pathway/metabolites and EMT. ACC=acetyl-CoA carboxylase; ACL=ATP citrate lyase; ACSL=acetyl-CoA synthetase; ALDOA=aldolase A; CaN=calcineurin A; CI-CV=respiratory chain complexes I-V; CoQ=coenzyme Q; CS=citrate synthase; CytC=cytochrome C; FBP1=fructose-1,6-bisphosphatase 1; FH=fumarate hydratase; GAPDH=glyceraldehyde-3-phosphate dehydrogenase; GLS=glutaminase; IDH=isocitrate dehydrogenase; LDHA=lactic dehydrogenase A; PGI =phosphoglucose isomerase; PKM2=pyruvate kinase M2; SCD=steroyl-CoA desaturase; SDH=succinate dehydrogenase.

Fig.3 EMT activation by mutations in *FH*, *SDH* and *IDH* requires epigenetic reprogramming.

Schematic representation of how mitochondrial metabolites accumulated upon mutation of the indicated TCA cycle enzymes activate the EMT. A common pathway affected by these metabolites is the epigenetic suppression of a family of antimetastatic microRNAs, miR200, *via* the inhibition of histone demethylases (KDMs) and DNA demethylases (TETs). Of note, in the case of 2HG, the suppression of miR200 is indirect, and occurs via activation of Zeb1/2. See the text for more details. FH=fumarate hydratase; SDH=succinate dehydrogenase; IDH=isocitrate dehydrogenase.

Fig.4 Integration between oncogenic signalling, metabolic transformation, and epigenetic reprogramming during EMT

EMT requires the coordinated activation of multiple cellular processes, here represented as gears within a clockwork. Each of these components are essential for the full activation of EMT. As consequence, the inhibition of parts of this clockwork hampers the full activation of the EMT. For

724 instance, inhibition of mutant IDH, or activation of PKA can block EMT. PKA=protein kinase A;

725 IDH=isocitrate dehydrogenase.

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