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Hervé Acloque, Jing Yang, Eric Theveneau

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1 Epithelial-to-Mesenchymal Plasticity from development to disease: an
2 introduction to the special issue.

3 Hervé Acloque¹, Jing Yang² and Eric Theveneau^{3*}

4 1. Université Paris Saclay, INRAE, AgroParisTech, GABI, Domaine de Vilvert, 78350 Jouy en Josas, France

5 2. Department of Pharmacology and of Pediatrics, Moores Cancer Center, University of California, San
6 Diego, School of Medicine, La Jolla, CA 92093, USA

7 3. Molecular, Cellular and Developmental Biology department (MCD), Centre de Biologie Intégrative
8 (CBI), Université de Toulouse, CNRS, UPS, 118 route de Narbonne 31062, Toulouse Cedex 09, France.

9 *corresponding author: eric.theveneau@univ-tlse3

10

11 **Abstract**

12 Epithelial-Mesenchymal Transition (EMT) refers to the ability of cells to switch between
13 epithelial and mesenchymal states, playing critical roles in embryonic development, wound healing,
14 fibrosis, and cancer metastasis. Here, we discuss some examples that challenge the use of specific
15 markers to define EMT, noting that their expression may not always correspond to the expected
16 epithelial or mesenchymal identity. In concordance with recent development in the field, we emphasize
17 the importance of generalizing the use of the term Epithelial-Mesenchymal Plasticity (EMP), to better
18 capture the diverse and context-dependent nature of the bidirectional journey that cells can undertake
19 between the E and M phenotypes. We highlight the usefulness of studying a wide range of physiological
20 EMT scenarios, stress the value of the dynamic of expression of EMP regulators and advocate,
21 whenever possible, for more systematic functional assays to assess cellular states.

22

23 **Main Text**

24 The term “Epithelial-Mesenchymal Transformation” was introduced by Elizabeth Hay to define
25 the phenomena that cells can switch from epithelial to mesenchymal states during tissue
26 morphogenesis and homeostasis. The term “transformation” is later replaced by the more accurate
27 word “transition” to better reflect the non-binary nature of the process (see (Hay, 2005) and references
28 therein). EMT is indeed a staple of embryonic development as cells must proliferate and move in three
29 dimensions to form and rearrange tissues and organs at the right place and time. For that, they
30 frequently toggle between relatively cohesive and stable epithelial states and more dynamic and loose
31 mesenchymal arrangements (Nieto et al., 2016). In many cases, and as discussed below, EMT occurs in

32 cells that will eventually migrate. However, one should not systematically associate EMT with migratory
33 behavior. During fibrosis, there are several examples of cells adopting partial E/M phenotypes that do
34 not undertake migration but nonetheless contribute to disease progression via this phenotypic change
35 (see (Liu et al., 2022) for discussion).

36 Classical examples of EMT include gastrulation and neural crest development. Mesoderm is
37 induced at the interface between ectoderm and endoderm. Initially epithelial, mesoderm progenitor
38 cells undergo a conversion towards mesenchymal states to ingress, migrate and intercalate between
39 ectoderm and endoderm during gastrulation. At the cervical and trunk level, the most axial of these
40 migratory mesodermal cells re-epithelialize into repetitive structures called somites on either sides of
41 the neural tube (Benazeraf & Pourquie, 2013). These somites will then undergo another round of
42 mesenchymalization at their ventral side to form the sclerotome, which later produces vertebrae, and
43 at their dorsal side to produce the dermis and muscle progenitors. Neural crest cells are multipotent
44 stem cells induced at the lateral border of the prospective central nervous system (Gouignard et al.,
45 2018). As neurulation proceeds, neural crest cells emerge from the neuroepithelium by converting into
46 highly migratory mesenchyme cells, many of which will later re-aggregate to form solid structures
47 ranging from condensed connective tissue, such as ganglia of the peripheral nervous system, to
48 epithelial cells of the corneal endothelium of the eye (Dupin et al., 2006). Other examples of EMT
49 during development occurs in the lateral mesoderm, the liver diverticulum, the pancreatic buds or the
50 endocardium (Lim & Thiery, 2012).

51 Importantly, EMT is not specific to embryonic development but also occurs during various
52 pathogenesis conditions, including wound healing, fibrosis and cancer metastasis (Yang et al., 2020).
53 Molecular and cellular mechanisms controlling EMT are evolutionally conserved due to their
54 physiological importance. Therefore, these settings provide knowledge databases on how EMT is
55 controlled, what signals may trigger EMT and how cells change their E/M characteristics over time. The
56 range of EMT possibilities and variations is huge. For instance, gastrulation occurs in all animals, except
57 Porifera (sponges) and Placozoa (Lanna, 2015; Martindale, 2005). While the basic principles are
58 conserved, EMT during gastrulation happens at different initial conditions in each species (i.e number
59 of cells, topology etc.). As for neural crest delamination, there are variations from species to species
60 but also among neural crest subpopulations in each animal. Therefore, by studying the wide diversity
61 of physiological EMTs in multiple experimental models, we can build a catalogue of various possible
62 scenarios for cells to undergo EMT and its reverse transition, MET.

63 We all hope for the right marker (or set of markers) whose expression could discriminate
64 between cells that have not yet undergone EMT and the ones that are engaged in EMT. Ideally at the

65 earliest possible moment, so that these expressions might have predictive value regarding to what cells
66 will do next. However, the overwhelming diversity of EMTs in physiological settings suggests that the
67 search for the right markers may be a wild goose chase. This is why we think that the previously
68 suggested term “Epithelial-Mesenchymal Plasticity (EMP)” (Haerinck et al., 2023; Yang et al., 2020)
69 should be used to define the framework within which cells’ journeys around E and M status are studied.
70 This makes it possible to think about and address plasticity between epithelial and mesenchymal cell
71 states as a whole without being limited to the simplistic and reductive definitions often linked to the
72 EMT/MET terminologies. The terms EMT and MET are still valid but they represent specific cases of
73 unidirectional changes between E and M that take place at a smaller time scale within the global long-
74 term EMP context. This framework puts less pressure on the use of markers as proof of a cell status
75 and more importance on cell behavior and overall capabilities of cells to interact with one another.

76 One telling example is that of cadherins. The expressions of these calcium-dependent cell
77 adhesion molecules are often used as definitive markers of epithelial versus mesenchymal identities
78 such that cells expressing E-cadherin (Cadherin-1, CDH1) are considered epithelial while those
79 expressing N-cadherin (Cadherin-2, CDH2) are thought to be mesenchymal. However, neural crest
80 development gives a different perspective on this idea. In *Xenopus*, pre-migratory cephalic neural crest
81 cells are epithelial and turn into mesenchymal migratory cells via an E to N cadherin switch (Scarpa et
82 al., 2015). However, it should be noted that these cells maintain some expression of E-cadherin (Huang
83 et al., 2016). During migration of *Xenopus* neural crest cells, E-cadherin is no longer involved in cell-cell
84 junctions and its loss of function impairs adhesion to fibronectin (Huang et al., 2016). Interestingly, the
85 role of maintaining cell-cell junctions during migration is performed by N-cadherin whose loss-of-
86 function impairs cell-cell adhesion (Theveneau et al., 2010). Therefore, *Xenopus* neural crest cells
87 display an intermediate E/M phenotype but this is due to the fact that these cells maintain some
88 transient junctions while migrating, not because they have some residual level of E-cadherin
89 expression. In other cell types, E-cadherin has been shown to be involved in cell-cell adhesions allowing
90 collective motion (Bazellieres et al., 2015). By contrast, in chicken embryos, cephalic neural crest cells
91 emerge from a neuroepithelium that expresses both E and N-cadherin. These cells become
92 mesenchymal and initiate migration while expressing E and N-cadherin (Dady et al., 2012; Dady &
93 Duband, 2017; Rogers et al., 2018; Theveneau et al., 2007). Whereas, at trunk level, chicken neural
94 crest cells depart from a neuroepithelium that only expresses N-cadherin and their
95 mesenchymalization occurs while maintaining N-cadherin expression (Shoval et al., 2007). These three
96 examples demonstrate that mesenchymalization of neural crest cells can occur concomitantly with an
97 upregulation, a stable expression or a loss of N-cadherin expression at mRNA level and a subsequent
98 complex dynamics at protein level. Thus, the change from E-to-M in neural crest cells cannot be simply

99 attributed to a cadherin switch. Similar observations can be made in other cell types. In gastrulating
100 paraxial mesoderm, N-cadherin is used for epithelialization of the somites from the mesenchymal
101 presomitic mesoderm (Chal et al., 2017). In adults, N-cadherin is endogenously expressed in multiple
102 organs under normal conditions (i.e liver, testis, adrenal gland and cardiomyocytes). These different
103 examples highlight the fact that high expression levels of N-cadherin at mRNA level may correspond to
104 very different situations in terms of stability of the N-cadherin protein and cell behavior (i.e. migration,
105 active epithelialization or stable differentiated organ). This is true for the neuroepithelium and
106 migratory neural crest cells. In the former, N-cadherin is involved in stable junctions and maintains the
107 epithelium. In the latter, the N-cadherin protein is cleaved and endocytosed, thus preventing neural
108 crest cells from forming stable cell-cell adhesions (Kuriyama et al., 2014; Shoval et al., 2007). Overall,
109 this indicates that expressions of cadherins by themselves are not indicative of a mesenchymal or
110 epithelial status and that the context in which these expressions occur has to be taken into account.

111 Another example is that of transcription factors that act upstream of the EMP programs during
112 development such as Snai, Twist, and Zeb family members. Developmental studies highlighted the
113 critical roles that many of these genes play in destabilizing epithelial features by repressing cadherin
114 expressions, upregulating proteases and modifying extracellular matrix. When examining EMP
115 transcription factors in the Human Protein Atlas (Human Protein Atlas proteinatlas.org; (Karlsson et al.,
116 2021; Uhlen et al., 2015)), one cannot help noticing that several of them also have normal expression
117 in certain cell types. One extreme example is that of the specialized epithelial cells of the male gonads,
118 known as Sertoli cells. These cells have an endogenous expression of Twist1, Zeb1, MMP14 and N-
119 cadherin. By all measures, a cell expressing simultaneously these four proteins would be considered a
120 highly invasive migratory cell by most developmental and cancer biologists. Yet, Sertoli cells are
121 epithelial and non-migratory. Another example is the expression of Snai1 and Snai2 in breast
122 adipocytes, cells with limited migratory potential under normal physiological conditions. The same
123 observation can be made for metalloproteinases whose expression is often used as a sign of invasive
124 behavior. While such enzymes can degrade extracellular matrix, generate tracks for migration and
125 invasion, numerous epithelia express metalloproteinases without displaying signs of EMP. During
126 neural tube development, MMP14 mRNA is strongly detected throughout the entire tissue (Andrieu et
127 al., 2020). However, MMP14-dependent EMP only occurs in the neural crest (Andrieu et al., 2020) while
128 the rest of the neural tube maintains an epithelial organization and a continuous basement membrane.
129 Therefore, while MMPs are involved in invasion, expression of MMPs alone cannot define invasiveness,
130 a trait only assessed via functional assays such as assessing motility, matrix degradation or the ability
131 to intermix with other cells. Finally, in some cell types E-cadherin is co-expressed with known repressors
132 of its expression under normal circumstances. Such examples include the co-expression of E-cadherin

133 and Snai2 in migratory cephalic neural crest cells in chicken embryos (Rogers et al., 2018). This shows
134 that levels of regulation other than gene expression, post-transcriptionally and post-translationally, are
135 relevant as well.

136 What do we learn from all of this? First, that the dynamic of expression, taking into
137 consideration the actual protein level, is likely to be more informative than a single measure at the RNA
138 level. Unfortunately, expression analyses at multiple time points in pathological contexts is often
139 technically challenging to achieve and/or initial control expression level in each patient/tissue may not
140 be known. When possible, such analyses may yield seemingly surprising results if only a small set of
141 factors is considered, such as the described reduction of Snai2 expression in malignant prostate cancer
142 compared to normal prostate (Esposito et al., 2015). If taken *stricto sensu* in the classical EMT
143 framework, such loss of Snai2 expression may be interpreted as an absence of conversion from E to M
144 associated with prostate cancer progression. A more likely situation is that of a progressive change of
145 transcription factor signature as seen in melanoma where the progression from proliferation to
146 invasion is associated with a change from Snai2/Zeb2 to a Twist1/Zeb1 profile (Caramel et al., 2013).
147 Interestingly, such transitions are also observed in neural crest development. In *Xenopus* cephalic
148 neural crest cells, Snai1/Snai2 are expressed in pre-migratory cells (Aybar et al., 2003). Then, several
149 hours later, Twist expression starts (Hopwood et al., 1989). As migration proceeds, Twist expression is
150 maintained and increased while those of Snai1 and 2 are lost. Functional evidence further indicate that
151 Twist physically interact with Snail proteins to inhibit their function (Lander et al., 2013). Second, that
152 the context plays a crucial part in interpreting the data. EMP gene expression in cells that do or do not
153 express such genes under normal physiological conditions will not carry the same weight. Third, part
154 of that context may be the subcellular localization of some of the putative EMP regulators themselves.
155 In the case of metalloproteinases, while they obviously can affect the matrix, they need to be presented
156 at the cell surface or released extracellularly to do so in the first place. We now know that many of such
157 proteins have complex subcellular trafficking and can be kept intracellularly (Jobin et al., 2017), in some
158 cases to promote EMP, as seen in *Xenopus* neural crest (Gougnard et al., 2023). Transcription factors
159 also traffic between the cytosol and preventing entry into the nucleus can block their function as shown
160 for TWIST1 in response to different extracellular matrix rigidities (Fattet et al., 2020; Wei et al., 2015).
161 Fourth, some EMP regulators may have additional functions beyond regulating EMP. Snail for instance
162 can influence cancer progression without triggering E-cadherin downregulation and EMP (Paul et al.,
163 2023) and Snai2 is required for normal hematopoiesis (Pioli & Weis, 2014). Twist1 is known as an
164 important factor for mesoderm development and differentiation most likely by modulating FGF and
165 Shh signaling as well as Hand proteins (Qin et al., 2012) but some of these targets might still be related
166 to EMP. Overexpression of Sox10 is sufficient to promote partial or complete mesenchymalization in

167 the neuroepithelium (McKeown et al., 2005). However, during normal neural crest development it
168 promotes pigment cell formation (Aoki et al., 2003) whereas its inhibition does not affect EMT and
169 migration (Aoki et al., 2003; Honore et al., 2003). Therefore, in some cases, expression of some EMP
170 genes may be related to cell identity/lineage rather than cell behavior. These observations require us
171 to rethink the framework associated with the initial definition of EMT by proposing a more flexible
172 paradigm, where the plasticity between E and M states is tissue and context-dependent and cannot be
173 reduced to a few key markers.

174 These observations stress the importance of monitoring expression of putative EMP regulators
175 across multiple time points and to perform functional assays to assess the cells' EMP state and
176 potential. In the context of cell and developmental biology studies multiple assays can be (and often
177 are) implemented alongside expression analyses: migration/invasion assays, cell-cell and cell-matrix
178 adhesion assays, collision assays, matrix remodeling/degradation assays etc... Given the wealth of
179 information that can be extracted from such experiments it would be greatly beneficial if the
180 diagnosis/prognosis workflow in the clinical context could integrate such approaches as routine
181 procedures from patients' biopsies. Currently, oncology centers in which there is a functional daily
182 integration of clinical and research departments are the exception rather than the norm. There have
183 been promising attempts to harness the power of classical embryology techniques in the context of
184 oncology via, for instance, modified migration/invasion assays using patients' cells grafted in avian
185 embryos (Delloye-Bourgeois et al., 2017; Jarrosson et al., 2021; Jarrosson et al., 2023) . Still very
186 marginal a few years ago, the use of these chimeras is expanding, both in the academic world and
187 biotech companies. Organoid development, from embryonic or adult stem cells, may also help to
188 recapitulate the features and dynamic of EMP during organogenesis, in physiology and pathologies. It
189 allows to access and assess processes that may be difficult to observe and to quantify either *in vivo* or
190 *in vitro* in 2D cell culture systems.

191 When people discuss EMP from development to diseases, they usually mean that EMP can be
192 found in a large spectrum of biological systems from the most physiological (i.e., development, healing)
193 all the way up to pathological settings (i.e., fibrosis and cancer). This, however, maintains the various
194 fields of investigation on parallel trajectories with researchers comparing systems, drawing similarities,
195 and searching for correlations. But we may be ignoring another level of analysis that could be more
196 relevant. Should we actually follow cells from development to disease? Thus, considering the life of
197 cells from development to normal homeostasis to the pathology? Organs are composed of cells that
198 have a history through which they have acquired a given gene expression profile, a given morphology
199 and a given set of interactions with their neighboring cells in their organs and with adjacent organs.
200 While some organs are formed from cells that never underwent EMP (epidermis), other went through

201 one (i.e. ganglia of the peripheral nervous system), two (i.e. dermis, skeletal muscle) or three (i.e.
202 cushion mesenchyme of the heart) round trips between epithelial and mesenchymal states. Should we
203 treat the variations in gene expression profiles and protein levels differently when dealing with cells
204 from various tissues that experienced one, two, three or no EMT at all throughout their lives? Or when
205 considering cells that display different endogenous expression of genes with EMP potential? Plasticity
206 around E/M states is known to impact stemness and survival, in addition to migratory and invasive
207 properties. Are cells durably affected by a chronic exposure to signals triggering EMP? Genome-scale
208 epigenetic modifications have been documented during EMP (Malouf et al., 2013; McDonald et al.,
209 2011). Do they have long-lasting effects in terms of competence to toggle between E, M and
210 intermediate states? We hope that future systematic studies could address the long term effects (if
211 any) of successive EMT-MET events on cells and their putative impact on subsequent EM plasticity
212 events.

213 This Editorial only scratches the surface of the complexity of EMP and the multiple questions it
214 raises. EMP is a rapidly expanding field of research and our understanding of its molecular and cellular
215 implementation as well as its functional relationship with normal and pathological processes is a work
216 in progress. In this special issue, we have assembled a collection of reviews and research articles looking
217 at EMP in a wide range of contexts such as lateral plate mesoderm and neural crest development,
218 cancer cell dormancy or kidney fibrosis. We hope that readers will find the content of the Special Issue
219 to be intellectually stimulating.

220

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228

229 **References**

230 Andrieu, C., Montigny, A., Bibonne, A., Despin-Guitard, E., Alfandari, D., & Theveneau, E. (2020).
231 MMP14 is required for delamination of chick neural crest cells independently of its catalytic
232 activity. *Development*, 147(7). <https://doi.org/10.1242/dev.183954>

233 Aoki, Y., Saint-Germain, N., Gyda, M., Magner-Fink, E., Lee, Y. H., Credidio, C., & Saint-Jeannet, J. P.
234 (2003). Sox10 regulates the development of neural crest-derived melanocytes in *Xenopus*. *Dev*
235 *Biol*, 259(1), 19-33. <http://www.ncbi.nlm.nih.gov/pubmed/12812785>

236 Aybar, M. J., Nieto, M. A., & Mayor, R. (2003). Snail precedes slug in the genetic cascade required for
237 the specification and migration of the *Xenopus* neural crest. *Development*, 130(3), 483-494.
238 <http://www.ncbi.nlm.nih.gov/pubmed/12490555>

239 Bazellieres, E., Conte, V., Elosegui-Artola, A., Serra-Picamal, X., Bintanel-Morcillo, M., Roca-Cusachs, P.,
240 . . . Trepas, X. (2015). Control of cell-cell forces and collective cell dynamics by the intercellular
241 adhesome. *Nat Cell Biol*, 17(4), 409-420. <https://doi.org/10.1038/ncb3135>

242 Benazeraf, B., & Pourquie, O. (2013). Formation and segmentation of the vertebrate body axis. *Annu*
243 *Rev Cell Dev Biol*, 29, 1-26. <https://doi.org/10.1146/annurev-cellbio-101011-155703>

244 Caramel, J., Papadogeorgakis, E., Hill, L., Browne, G. J., Richard, G., Wierinckx, A., . . . Tulchinsky, E.
245 (2013). A switch in the expression of embryonic EMT-inducers drives the development of
246 malignant melanoma. *Cancer Cell*, 24(4), 466-480. <https://doi.org/10.1016/j.ccr.2013.08.018>

247 Chal, J., Guillot, C., & Pourquie, O. (2017). P APC couples the segmentation clock to somite
248 morphogenesis by regulating N-cadherin-dependent adhesion. *Development*, 144(4), 664-676.
249 <https://doi.org/10.1242/dev.143974>

250 Dady, A., Blavet, C., & Duband, J. L. (2012). Timing and kinetics of E- to N-cadherin switch during
251 neurulation in the avian embryo. *Dev Dyn*, 241(8), 1333-1349.
252 <https://doi.org/10.1002/dvdy.23813>

253 Dady, A., & Duband, J. L. (2017). Cadherin interplay during neural crest segregation from the non-neural
254 ectoderm and neural tube in the early chick embryo. *Dev Dyn*, 246(7), 550-565.
255 <https://doi.org/10.1002/dvdy.24517>

256 Delloye-Bourgeois, C., Bertin, L., Thoinet, K., Jarrosson, L., Kindbeiter, K., Buffet, T., . . . Castellani, V.
257 (2017). Microenvironment-Driven Shift of Cohesion/Detachment Balance within Tumors
258 Induces a Switch toward Metastasis in Neuroblastoma. *Cancer Cell*, 32(4), 427-443 e428.
259 <https://doi.org/10.1016/j.ccell.2017.09.006>

260 Dupin, E., Creuzet, S., & Le Douarin, N. M. (2006). The contribution of the neural crest to the vertebrate
261 body. *Adv Exp Med Biol*, 589, 96-119. https://doi.org/10.1007/978-0-387-46954-6_6

262 Esposito, S., Russo, M. V., Airoidi, I., Tupone, M. G., Sorrentino, C., Barbarito, G., . . . Di Carlo, E. (2015).
263 SNAI2/Slug gene is silenced in prostate cancer and regulates neuroendocrine differentiation,
264 metastasis-suppressor and pluripotency gene expression. *Oncotarget*, 6(19), 17121-17134.
265 <https://doi.org/10.18632/oncotarget.2736>

266 Fattet, L., Jung, H. Y., Matsumoto, M. W., Aubol, B. E., Kumar, A., Adams, J. A., . . . Yang, J. (2020). Matrix
267 Rigidity Controls Epithelial-Mesenchymal Plasticity and Tumor Metastasis via a
268 Mechanoresponsive EPHA2/LYN Complex. *Dev Cell*, 54(3), 302-316 e307.
269 <https://doi.org/10.1016/j.devcel.2020.05.031>

270 Gougnard, N., Andrieu, C., & Theveneau, E. (2018). Neural crest delamination and migration: Looking
271 forward to the next 150 years. *Genesis*, e23107. <https://doi.org/10.1002/dvg.23107>

272 Gougnard, N., Bibonne, A., Mata, J. F., Bajanca, F., Berki, B., Barriga, E. H., . . . Theveneau, E. (2023).
273 Paracrine regulation of neural crest EMT by placodal MMP28. *PLoS Biol*, 21(8), e3002261.
274 <https://doi.org/10.1371/journal.pbio.3002261>

275 Haerincx, J., Goossens, S., & Berx, G. (2023). The epithelial-mesenchymal plasticity landscape:
276 principles of design and mechanisms of regulation. *Nat Rev Genet*, 24(9), 590-609.
277 <https://doi.org/10.1038/s41576-023-00601-0>

278 Hay, E. D. (2005). The mesenchymal cell, its role in the embryo, and the remarkable signaling
279 mechanisms that create it. *Dev Dyn*, 233(3), 706-720. <https://doi.org/10.1002/dvdy.20345>

280 Honore, S. M., Aybar, M. J., & Mayor, R. (2003). Sox10 is required for the early development of the
281 prospective neural crest in *Xenopus* embryos. *Dev Biol*, 260(1), 79-96.
282 <http://www.ncbi.nlm.nih.gov/pubmed/12885557>

283 Hopwood, N. D., Pluck, A., & Gurdon, J. B. (1989). A *Xenopus* mRNA related to *Drosophila* twist is
284 expressed in response to induction in the mesoderm and the neural crest. *Cell*, *59*(5), 893-903.
285 <https://www.ncbi.nlm.nih.gov/pubmed/2590945>

286 Huang, C., Kratzer, M. C., Wedlich, D., & Kashef, J. (2016). E-cadherin is required for cranial neural crest
287 migration in *Xenopus laevis*. *Dev Biol*, *411*(2), 159-171.
288 <https://doi.org/10.1016/j.ydbio.2016.02.007>

289 Jarrosson, L., Costechareyre, C., Gallix, F., Cire, S., Gay, F., Imbaud, O., . . . Castellani, V. (2021). An avian
290 embryo patient-derived xenograft model for preclinical studies of human breast cancers.
291 *iScience*, *24*(12), 103423. <https://doi.org/10.1016/j.isci.2021.103423>

292 Jarrosson, L., Dalle, S., Costechareyre, C., Tang, Y., Grimont, M., Plaschka, M., . . . Delloye-Bourgeois, C.
293 (2023). An in vivo avian model of human melanoma to perform rapid and robust preclinical
294 studies. *EMBO Mol Med*, *15*(3), e16629. <https://doi.org/10.15252/emmm.202216629>

295 Jobin, P. G., Butler, G. S., & Overall, C. M. (2017). New intracellular activities of matrix
296 metalloproteinases shine in the moonlight. *Biochim Biophys Acta Mol Cell Res*, *1864*(11 Pt A),
297 2043-2055. <https://doi.org/10.1016/j.bbamcr.2017.05.013>

298 Karlsson, M., Zhang, C., Mear, L., Zhong, W., Digre, A., Katona, B., . . . Lindskog, C. (2021). A single-cell
299 type transcriptomics map of human tissues. *Sci Adv*, *7*(31).
300 <https://doi.org/10.1126/sciadv.abh2169>

301 Kuriyama, S., Theveneau, E., Benedetto, A., Parsons, M., Tanaka, M., Charras, G., . . . Mayor, R. (2014).
302 In vivo collective cell migration requires an LPAR2-dependent increase in tissue fluidity. *J Cell*
303 *Biol*, *206*(1), 113-127. <https://doi.org/10.1083/jcb.201402093>

304 Lander, R., Nasr, T., Ochoa, S. D., Nordin, K., Prasad, M. S., & Labonne, C. (2013). Interactions between
305 Twist and other core epithelial-mesenchymal transition factors are controlled by GSK3-
306 mediated phosphorylation. *Nat Commun*, *4*, 1542. <https://doi.org/10.1038/ncomms2543>

307 Lanna, E. (2015). Evo-devo of non-bilaterian animals. *Genet Mol Biol*, *38*(3), 284-300.
308 <https://doi.org/10.1590/S1415-475738320150005>

309 Lim, J., & Thiery, J. P. (2012). Epithelial-mesenchymal transitions: insights from development.
310 *Development*, *139*(19), 3471-3486. <https://doi.org/10.1242/dev.071209>

311 Liu, L., Sun, Q., Davis, F., Mao, J., Zhao, H., & Ma, D. (2022). Epithelial-mesenchymal transition in organ
312 fibrosis development: current understanding and treatment strategies. *Burns Trauma*, *10*,
313 tkac011. <https://doi.org/10.1093/burnst/tkac011>

314 Malouf, G. G., Taube, J. H., Lu, Y., Roysarkar, T., Panjarian, S., Estecio, M. R., . . . Issa, J. P. (2013).
315 Architecture of epigenetic reprogramming following Twist1-mediated epithelial-mesenchymal
316 transition. *Genome Biol*, *14*(12), R144. <https://doi.org/10.1186/gb-2013-14-12-r144>

317 Martindale, M. Q. (2005). The evolution of metazoan axial properties. *Nat Rev Genet*, *6*(12), 917-927.
318 <https://doi.org/10.1038/nrg1725>

319 McDonald, O. G., Wu, H., Timp, W., Doi, A., & Feinberg, A. P. (2011). Genome-scale epigenetic
320 reprogramming during epithelial-to-mesenchymal transition. *Nat Struct Mol Biol*, *18*(8), 867-
321 874. <https://doi.org/10.1038/nsmb.2084>

322 McKeown, S. J., Lee, V. M., Bronner-Fraser, M., Newgreen, D. F., & Farlie, P. G. (2005). Sox10
323 overexpression induces neural crest-like cells from all dorsoventral levels of the neural tube but
324 inhibits differentiation. *Dev Dyn*, *233*(2), 430-444. <https://doi.org/10.1002/dvdy.20341>

325 Nieto, M. A., Huang, R. Y., Jackson, R. A., & Thiery, J. P. (2016). EMT: 2016. *Cell*, *166*(1), 21-45.
326 <https://doi.org/10.1016/j.cell.2016.06.028>

327 Paul, M. C., Schneeweis, C., Falcomata, C., Shan, C., Rossmeis, D., Koutsouli, S., . . . Saur, D. (2023). Non-
328 canonical functions of SNAIL drive context-specific cancer progression. *Nat Commun*, *14*(1),
329 1201. <https://doi.org/10.1038/s41467-023-36505-0>

330 Pioli, P. D., & Weis, J. H. (2014). Snail transcription factors in hematopoietic cell development: a model
331 of functional redundancy. *Exp Hematol*, *42*(6), 425-430.
332 <https://doi.org/10.1016/j.exphem.2014.03.002>

333 Qin, Q., Xu, Y., He, T., Qin, C., & Xu, J. (2012). Normal and disease-related biological functions of Twist1
334 and underlying molecular mechanisms. *Cell Res*, 22(1), 90-106.
335 <https://doi.org/10.1038/cr.2011.144>

336 Rogers, C. D., Sorrells, L. K., & Bronner, M. E. (2018). A catenin-dependent balance between N-cadherin
337 and E-cadherin controls neuroectodermal cell fate choices. *Mech Dev*, 152, 44-56.
338 <https://doi.org/10.1016/j.mod.2018.07.003>

339 Scarpa, E., Szabo, A., Bibonne, A., Theveneau, E., Parsons, M., & Mayor, R. (2015). Cadherin Switch
340 during EMT in Neural Crest Cells Leads to Contact Inhibition of Locomotion via Repolarization
341 of Forces. *Dev Cell*, 34(4), 421-434. <https://doi.org/10.1016/j.devcel.2015.06.012>

342 Shoval, I., Ludwig, A., & Kalcheim, C. (2007). Antagonistic roles of full-length N-cadherin and its soluble
343 BMP cleavage product in neural crest delamination. *Development*, 134(3), 491-501.
344 <https://doi.org/10.1242/dev.02742>

345 Theveneau, E., Duband, J. L., & Altabef, M. (2007). Ets-1 confers cranial features on neural crest
346 delamination. *PLoS One*, 2(11), e1142. <https://doi.org/10.1371/journal.pone.0001142>

347 Theveneau, E., Marchant, L., Kuriyama, S., Gull, M., Moeppps, B., Parsons, M., & Mayor, R. (2010).
348 Collective chemotaxis requires contact-dependent cell polarity. *Dev Cell*, 19(1), 39-53.
349 <https://doi.org/10.1016/j.devcel.2010.06.012>

350 Uhlen, M., Fagerberg, L., Hallstrom, B. M., Lindskog, C., Oksvold, P., Mardinoglu, A., . . . Ponten, F.
351 (2015). Proteomics. Tissue-based map of the human proteome. *Science*, 347(6220), 1260419.
352 <https://doi.org/10.1126/science.1260419>

353 Wei, S. C., Fattet, L., Tsai, J. H., Guo, Y., Pai, V. H., Majeski, H. E., . . . Yang, J. (2015). Matrix stiffness drives
354 epithelial-mesenchymal transition and tumour metastasis through a TWIST1-G3BP2
355 mechanotransduction pathway. *Nat Cell Biol*, 17(5), 678-688.
356 <https://doi.org/10.1038/ncb3157>

357 Yang, J., Antin, P., Berx, G., Blanpain, C., Brabletz, T., Bronner, M., . . . Association, E. M. T. I. (2020).
358 Guidelines and definitions for research on epithelial-mesenchymal transition. *Nat Rev Mol Cell*
359 *Biol*, 21(6), 341-352. <https://doi.org/10.1038/s41580-020-0237-9>

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