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Hijacking a morphogenesis proteinase for cancer cell invasion

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Abstract:

A long-standing question in biology is whether cancer cells exploit developmental processes to invade the surrounding stroma. In this issue of *Developmental Cell*, Feinberg *et al.* (2018), identify the matrix metalloproteinase Mmp14 as a key driver of mammary invasion common to both development and cancer, but expose an underlying twist.

The mammary gland is one of the few organs in the body that undergoes most of its development post-natally. Its growth is primarily activated in adolescent females where epithelial tubes rapidly sprout into a ductal tree. Epithelia within the mammary gland are also precursors of breast cancer, a disease that only develops in adulthood. Mammary ducts are compartmentalised into a bi-layer of inner luminal epithelia and outer myoepithelia. At their ends is a specialized structure called the terminal end bud, containing a reservoir of cell types required for duct elongation. Each tube is covered by a laminin-rich basement membrane (BM) embedded in a fibrous collagen matrix with stromal cells and surrounded by adipose tissue (Figure 1a). Post-natal duct extension in the developing mammary gland requires invasion through the stromal matrix and fat pad; likewise breast cancer cells invade the BM and stroma to spread to other parts of the body (Cheung *et al.*, 2013). A long-standing question is whether they use the same components and mechanisms. Part of the problem is that we do not fully understand the processes involved in the tightly controlled developmental invasion. In this issue of *Developmental Cell*, Feinberg and colleagues (Feinberg *et al.*, 2018) take advantage of the unique post-natal growth and remodelling properties of the mammary gland to identify a common component of tissue invasive programs utilized in branching morphogenesis and in neoplastic invasion, revealing an unexpected twist.

Controlled degradation and remodelling of the extracellular matrix (ECM) by matrix metalloproteinases (MMPs) is essential for branching morphogenesis (Khokha and Werb, 2011). Feinberg *et al.* (2018) focussed on two membrane-bound MMPs, Mmp14 and Mmp15, that degrade both BM and stromal ECM components (Feinberg *et al.*, 2016). Using elegant *in vivo* approaches, they first showed that both of these proteinases are present in the growing duct epithelium. Unexpectedly however, removal of either Mmp14 or Mmp15 in epithelial cells did not perturb duct outgrowth. This came as a surprise as Mmp14 has previously been shown to regulate branching morphogenesis *in vitro* (Feinberg *et al.*, 2016; Mori *et al.*, 2013). Branching morphogenesis also proceeded normally in mice lacking both epithelial specific Mmp14 and -15 excluding the possibility of redundant roles. Leaving no stone unturned, the authors then transplanted tiny bits of ducts lacking epithelial Mmp14 into cleared mammary fat pads of compatible females; these also branched out normally. By contrast, in a mouse mammary epithelial carcinoma model, epithelial targeting of Mmp14 but not Mmp15 blocked invasion and metastasis both *in vivo* and in organoid culture. The findings were reproduced in patient-derived xenografts, where blocking Mmp14 prevented human organoid invasion in collagen gels. Hence epithelial targeting of Mmp14 blocks invasion in cancer cells but not postnatal duct extension.

To understand this perplexing result, the authors decided to tamper with the proteinases in the periductal stromal compartment. Targeting stromal cell Mmp14 severely blocked post-

natal duct extension without altering duct architecture. In contrast, targeting Mmp14 in the stromal compartment of mammary carcinomas failed to block tumour cell invasion *in vivo*. Thus Mmp14 is a common factor that drives invasion in both developmental duct penetration and cancer cell invasion but the cellular compartments that control the Mmp14-mediated invasion are distinct. Post-natal duct growth and penetration through the ECM is tightly policed by the stromal fibroblasts, while cancer cells escape this regulation and appear to do the job of hacking through the ECM all by themselves (Figure 1b.c).

Why cancer cells exploit their cell-surface Mmp14 while healthy duct epithelia do not is not entirely clear. The ECM environment might contribute, given that duct epithelia are separated from the stromal matrix by a BM (rich in collagen IV) whereas cancer cells breach this barrier and directly touch the stromal ECM (rich in collagen I). Indeed cell-surface stabilisation of Mmp14 is regulated by collagen I ECM (Lafleur et al., 2006). Moreover, duct organoids, when embedded in collagen I hydrogels, manifest a dependency on Mmp14 for branching out but do not require this proteinase when embedded within a reconstituted BM (Feinberg et al., 2016). *In vivo*, thinning of the BM is commonly seen around the tips of rapidly growing ducts and tiny gaps in the BM do form, but whether the duct epithelia ever directly touch the stroma remains unclear. Regardless, contact with the stroma may not explain everything. In neoplasia, for example, Mmp14 may also be activated independently of stromal collagen I, as this proteinase has previously been shown to bestow cancer cells with the ability to degrade the BM (Ota et al., 2009). Furthermore, in the present study, tumours lacking Mmp14 have a thickened BM suggesting that at least some of the invasive activity might be halted at the cell-BM axis. Finally, Mmp14 also activates pro-Mmp2, which degrades BM components and is linked to breast cancer progression (Strongin et al., 1995).

Given that Mmp14 has both proteolytic and non-proteolytic roles. (Mori et al., 2013), the authors examined further the potential role of proteolysis by searching for evidence of stromal collagen remodelling. Using a collagen-hybridising peptide, the authors revealed collagen degradation proximal to the growing regions of mammary ducts. They also found increased periductal collagen in the stroma of Mmp14 null glands, suggesting loss of proteolytic ECM degradation. Moreover, branching morphogenesis failed to proceed in mutant mice expressing a non-degradable collagen I, similar to the stromal-depleted Mmp14. In both models, however, there was a severe block in proliferation of the epithelial and stromal cells, which probably accounts for the bulk loss of duct extension *in vivo*. Interestingly, Mmp2 and Mmp11 knockout mice also exhibit a similar defect in duct outgrowth (Wiseman et al., 2003).

These findings support a proteolytic role for Mmp14, but how ECM degradation is linked to post-natal duct extension remains unclear. In particular, if forward progression of ducts is blocked as a result of improper ECM remodelling, why do the epithelial tubes not continue to grow perpendicular to their long axis or into the lumen? It is thus possible that the proteolytic role of MMPs goes beyond just 'clearing the path' for invading ducts. It may also enable cell signalling, for example by releasing growth factors tethered to the ECM or exposing cryptic integrin binding sites (Sternlicht and Werb, 2001). Hence a block in this signalling axis could easily perturb tissue proliferation.

Another important finding in the present study is that a thickened periductal stromal matrix is not sufficient to support carcinoma cell invasion. Without Mmp14 the stromal matrix increased in density, but neither the tumours nor healthy breast cells were able to invade. Moreover, tumour organoids embedded in a non-degradable collagen I matrix *in vitro* displayed reduced invasion. Thus, a more dense stromal matrix is not sufficient to promote invasion without Mmp14 to help navigate through the ECM.

In conclusion, neoplasia can reactivate developmental molecules in invasive programs but not necessarily in an identical manner. At least for Mmp14, the job switches from one breast compartment to another.

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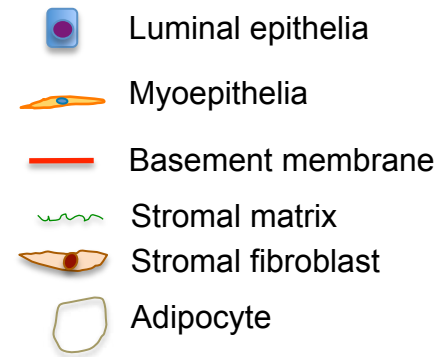
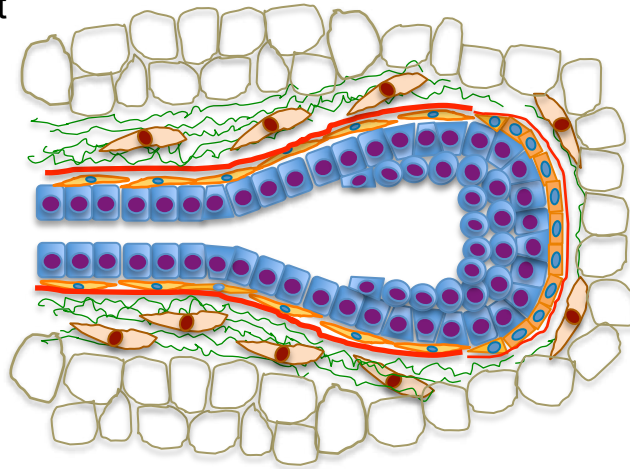
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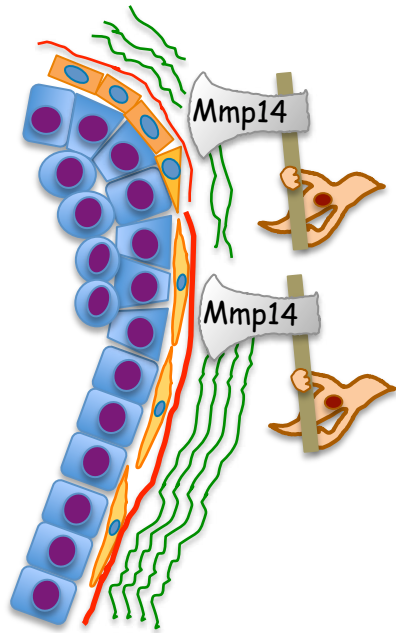
Figure 1: Extracellular matrix remodelling by Mmp14 in mammary gland development and cancer.

- a) Schematic of a mammary duct with a terminal end bud, depicting the epithelial and stromal compartments.
- b) In branching morphogenesis penetration through the ECM is tightly controlled by stromal fibroblast-derived Mmp14.
- c) Epithelial cancer cells utilize their own Mmp14 to invade through the ECM.

a) Mammary duct



b) Branching morphogenesis



c) Cancer cell invasion

