News and views on EMT Fra-1 controls EMT in mammary epithelial cells

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Abbreviations: AP-1, Activator Protein 1; EMT, Epithelial-to-mesenchymal transition; EMT-TF, Epithelial-to-mesenchymal transition transcription factor; Fra-1, Fos-related antigen 1; GEMMs, Genetically engineered mouse models; TF, transcription factor; TGFβ, Transforming growth factor β; Zeb1, Zinc finger E-box binding homeobox 1; Zeb2, Zinc finger E-box binding homeobox 2.

Epithelial-to-mesenchymal transition (EMT) is a complex biological program occurring in physiological and pathological conditions. The acquisition of a mesenchymal state by cancer cells is associated with loss of epithelial features, decreased cell adhesion and increased migration and invasion, which together are crucial for metastasis.

Down-regulation of the adherens junction protein E-cadherin is a hallmark of EMT. E-cadherin expression and epithelial plasticity are regulated by a number of molecular signaling pathways and regulators operating within dynamic circuits. Transcription factors (TFs) of the Snail, Twist and Zinc finger E-box binding homeobox (Zeb) families (collectively EMT-TFs) repress E-cadherin transcription and modulate additional epithelial and mesenchymal genes. EMT-TFs are controlled by growth factors such as Transforming growth factor β (TGFβ) and microRNAs, but also by a number of TFs.1,2

Fos-related antigen 1 (Fra-1) dimerize with Jun proteins to form the Activator Protein 1 (AP-1) transcription factor complex. Several studies, using human tumor material and cell lines correlated Fra-1 expression with malignancy. Compelling evidence implicates Fra-1 in EMT of human breast cancer cells and reports have connected Fra-1 as a dimer with c-JUN, with the EMT-TFs SNAI2/SLUG, ZEB1 and ZEB2.2,4

In our recent publication,5 we set out to define how Fra-1 contributes to EMT and assess the evolutionary conservation of EMT-TF regulation by Fra-1. We used the EpH4 murine non-tumourigenic mammary epithelial cells, which display a well documented phenotypic plasticity in response to oncogenes or growth factors. Ectopic expression of Fra-1 in EpH4 cells (EpFra1) was sufficient to trigger a proliferative, mesenchymal and invasive phenotype. Importantly, EpFra1 cells formed tumors in orthotopic and lung colonisation assays. Mechanistically, Fra-1 induced critical changes in the mRNA and protein expression of Tgfβ1 and 2 EMT-TFs: Zeb1 and Zeb2. Gene expression profiling confirmed that Fra-1 induced gene expression programmes characteristic of EMT, transformation and invasiveness. In addition, we identified a Zeb1/2 molecular signature in Fra-1-expressing cells, which was further validated by mRNA and protein analyses. Moreover, and in line with the recently emerging relationship between EMT, stemness and Fra-1/AP-1 in breast cancer,6 EpFra1 cells displayed a mammary stem cell signature. Finally, distinct changes in the expression of genes such as Secreted acidic cysteine rich glycoprotein (Sparc), Matrix metallopeptidase 2 (Mmp2), Interleukin 13 receptor, α 2 (Il13ra2), and Angiopoietin-like 4 (Angptl4), previously associated with lung tropism of human breast cancer cells,6 were measured.

We next determined how Fra-1 modulates Tgfβ1 and Zeb1/2 expression and how these genes contribute to the phenotypes observed in EpFra1 cells. Chromatin immunoprecipitation demonstrated that Fra-1 bound the mouse Tgfβ1 promoter. However, inhibiting TGFβ signaling in EpFra1 cells only modestly increased epithelial marker expression, indicating that TGFβ signaling contributes, but is likely not essential for Fra-1-induced EMT.

We next tested whether Fra-1 directly regulates Zeb1 and Zeb2 transcription, focusing on genomic sequences with...
potential AP-1-binding elements conserved in the human genes. Fra-1 bound the murine Zeb2 promoter and we identified 3 novel AP-1 sites responsible for the activation of a Zeb2 promoter reporter by Fra-1. This demonstrates for the first time, a direct transcriptional regulation of Zeb2 by an AP-1 TF. In addition, Fra-1 bound a region in the mouse Zeb1 gene homologous to the region of the human ZEB1 gene recently implicated in breast cancer cell plasticity. Whether FRA-1 regulates ZEB1 expression in breast cancer cells by altering the epigenetic landscape is an attractive possibility that certainly merits future investigation.

Consistent with the situation in human cells, RNA interference demonstrated that the EMT phenotype triggered by Fra-1 was largely dependent on increased Zeb1/2. Reducing the expression of Zeb1 and Zeb2, and to a lesser extent Zeb2 in EpFra1 cells, restored the expression of Zeb target genes including E-cadherin and reduced migration and invasion in vitro. In addition, EpFra1 cells with Zeb1/2 knock-down displayed decreased orthotopic tumourigenesis. However, most strikingly these cells could still colonize the lungs, with a notable fraction of the tumors retaining E-cadherin expression. Thus Zeb1 and Zeb2 are critical mediators of E-cadherin downregulation and EMT downstream of Fra-1, while other Fra-1 targets, independent of Zeb1/2, likely sustain the proliferative, tumourigenic and distant organ colonisation capacity of EpFra1 cells (Fig. 1).

Genetically engineered mouse models (GEMMs) are of great potential for therapy development, in particular to tackle the metastatic phase of cancer in a non-compromised immune environment. Our work provides novel insights how Fra-1/ AP-1 is connected to the complex network of EMT modulators and demonstrates that the mechanistic basis of Fra-1 contribution to EMT is largely conserved in mouse cells. The next step is to evaluate our findings in GEMMs and assess how manipulating Fra-1 expression in the tumor or its environment, would affect mammary tumor development in vivo. Transgenic mice broadly expressing Fra-1 develop osteosclerosis and generalized fibrosis, which precludes mammary tumor analyses. We recently generated switchable, cell type-specific, gain of function alleles for Fra-1 and for the c-Jun-Fra-1 dimer, which should allow to carefully assess the phenotypic consequences of increased Fra-1 expression in the mammary epithelium of wild-type or breast cancer-prone mice, such as the MMTV-PyMT GEMM. Conversely, the therapeutic potential of inactivating Fra-1 along the different stages of breast cancer progression could be assessed by inducible gene inactivation in specific cells of the mammary gland, a strategy we successfully applied in the past to liver cancer. The results from such studies will likely provide further valuable insights into how to best inhibit metastatic tumor progression and help improve the clinical outcome of cancer patients.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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