

# MicroRNA-dependent regulation of the microenvironment and the epithelial stromal cell interactions in the mouse mammary gland

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MicroRNAs are endogenous small RNAs regulating the expression of their target genes on the post-transcriptional level. Up to now, loss-of-function mutants were generated in mice for only a few individual microRNAs in order to elucidate their functions *in vivo*.<sup>1</sup> These studies showed that these individual microRNAs play important roles in both the cardiovascular and immune systems. We have recently shown that the miR-212/132 family has an indispensable function in the development of mammary gland in mice. The epithelial-stromal cell communications, necessary for the pubertal development of mammary gland, is regulated by the miR-212/132 family. This represents the first example for a microRNA function in a developmental process propagated through the organ microenvironment.<sup>2</sup>

Mammary gland development occurs in several distinct stages:<sup>3</sup> epithelial placode formation during embryogenesis; isometric growth of rudimentary ducts in the pre-pubertal stage; ductal outgrowth during puberty; lobuloalveolar differentiation during pregnancy; production and secretion of milk in lactation stage; and regression of the lobuloalveolar structures during the involution stage. Cell-cell and cell-matrix interactions, as well as endocrine and local growth factors regulate these processes. In miR-212/132 null female mice, embryonic and pre-pubertal mammary gland development appeared normal, whereas the ductal outgrowth during the pubertal stage was impaired.<sup>2</sup> However, the rudimentary tree in mutant mice, which did not grow and invade the fat pad during the pubertal stage, was still

able to differentiate into lobuloalveolar structures during pregnancy and secrete milk during the lactation phase.<sup>2</sup>

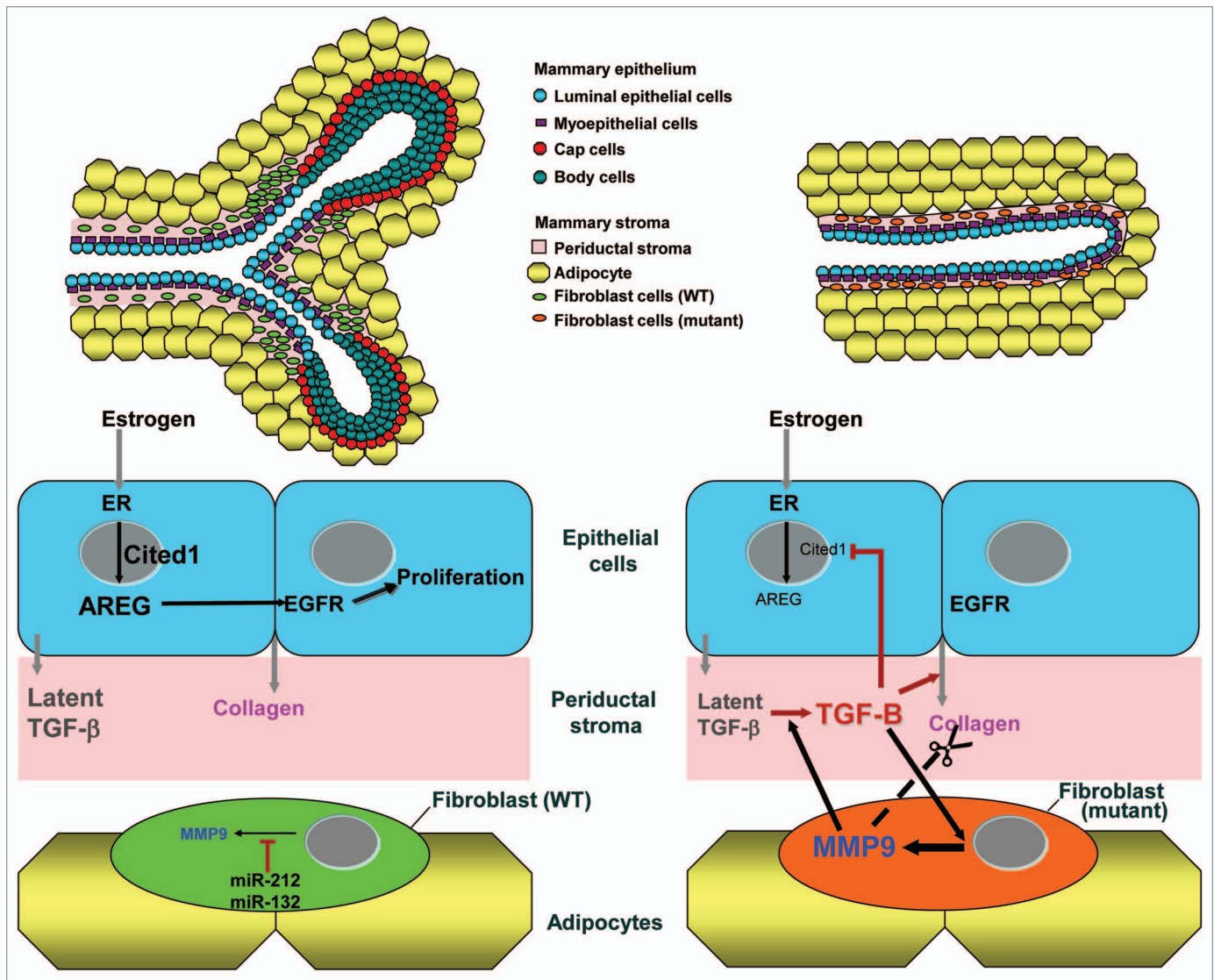
The mammary gland of virgin mice is composed of epithelial ductal structures and the surrounding stroma (Fig. 1). Epithelial ducts contain a single outer layer of myoepithelial cells and a single inner layer of luminal epithelial cells. Terminal end buds (TEBs) are specialized structures at the distal ends of the ducts, driving the ductal outgrowth during puberty. TEBs are composed of a single outer layer of cap cells and multilayered body cells. The mammary stroma comprises the periductal stroma and the fat pad. The periductal stroma, surrounding the epithelial ducts, is composed of fibroblasts and the collagen-rich extracellular matrix (ECM). The fat pad contains adipocytes, fibroblasts, preadipocytes, endothelial cells and mast cells.

Transplantation experiments were carried out in which wild-type mammary epithelial cells were introduced into fat pads of mutant mice, and vice versa. They clearly demonstrated that for the proper ductal outgrowth, miR-212/132 family expression is required in the stromal cells, but not in the epithelial cells.<sup>2</sup> Histological investigation of miR-212/132 null glands revealed a collagen deposition defect in the mutant stroma.<sup>2</sup> In wild-type glands, the growth-quiescent ducts are surrounded by the collagen-rich periductal stroma, which functions as a reservoir of epithelial cell growth factors. TGF $\beta$  is maintained in a latent state in the ECM of the periductal stroma in close proximity to the epithelial ducts.<sup>4</sup> Its interaction with ECM restricts

the access of enzymes to latent TGF $\beta$  and thus their activation potential.<sup>5</sup>

Both miR-212 and miR-132 are exclusively expressed in the mammary stroma and can directly regulate the expression of matrix metalloproteinase 9 (MMP-9), which is also mainly expressed by the mammary stroma.<sup>2</sup> In miR-212/132 null mammary glands, MMP-9 levels increase strongly and accumulate within the periductal stroma. Since MMP-9 has a high collagenase activity, the collagen deposition defect observed in the mutant glands can probably be ascribed to the increased abundance of MMP-9 within the stroma. Importantly, MMP-9 can also activate latent TGF $\beta$ .<sup>6</sup> Therefore, we propose that in the absence of collagen around the mutant ducts the latent TGF $\beta$  can be accessed and activated by MMP-9. TGF $\beta$  exerts its anti-proliferative function in epithelial cells by repressing the expression of CITED-1, which is required for the estrogen receptor (ER)-dependent amphiregulin (AREG) expression. The expression and secretion of collagen by epithelial cells is also upregulated by TGF $\beta$  in order to maintain the proper collagen deposition. Moreover, active TGF $\beta$  can also diffuse through the fat pad in the absence of collagen-rich periductal stroma and lead to the activation of MMP-9 transcription in stromal cells. This might generate a positive feedback loop, which increases the levels of MMP-9 protein and consequently the levels of TGF $\beta$  activity. This process is exacerbated in miR-212/132 null glands lacking the negative regulation of MMP-9 expression (Fig. 1). We propose that the function of miR-212/132 family in mammary

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**Figure 1.** Schematic representations of the ductal termini of wild-type (left) and miR-212/132 null mammary glands (right) and the proposed molecular mechanism for the functions of miR-212 and miR-132. Epithelial cells secrete collagen and latent TGF $\beta$  into the periductal stroma. The MMP-9 levels secreted by wild-type (WT) fibroblasts are restricted via the negative regulation of its expression by miR-212 and miR-132. Therefore, MMP-9 levels are insufficient to degrade collagen-rich ECM and activate latent TGF $\beta$ . In the absence of miR-212 and miR-132, the MMP-9 levels increase in the mutant stroma and lead to the degradation of collagen and activation of latent TGF $\beta$ . In epithelial cells, TGF $\beta$  suppresses the ER-dependent proliferative pathways by downregulation of CITED-1 expression. TGF $\beta$  also upregulates the synthesis of collagen in epithelial cells and MMP-9 in fibroblasts. This generates a positive feedback loop and leads to high levels of MMP-9 and TGF $\beta$  activities around the mutant epithelial ducts.

gland development is exerted by restricting the level of MMP-9 produced by stromal cells and the prevention of TGF $\beta$  pathway activation with its anti-proliferative consequences for epithelial cells.

Tamoxifen, which is an estrogen receptor antagonist being widely used in the treatment of breast cancer, leads to the upregulation of MMP-9 activity in breast tumors.<sup>7</sup> Interestingly, the collagen-dense breast tissue is associated with the increased

risk of breast cancer in humans.<sup>8,9</sup> These observations suggest a link between collagen deposition, MMP-9 activity and breast tumorigenesis. On the other hand, TGF $\beta$  is a very potent inhibitor of the proliferation of human mammary epithelial cells as well as of human breast cancer cell lines. It acts as a tumor suppressor for breast epithelia, at least in the early stages of tumorigenesis.<sup>10</sup> However, TGF $\beta$  can also promote the progression of breast cancer

in later stages by stimulating angiogenesis and inhibiting the anti-tumor immune responses.<sup>10</sup> Therefore, the positive feedback loop between the MMP-9 and TGF $\beta$  activities, which is negatively regulated by miR-212 and miR-132, might also indicate a possible involvement of miR-212/132 function in breast tumorigenesis. It will be important in future studies to define the functions of miR-212/132 in tumor-associated breast stroma.

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