Met signaling regulates growth, repopulating potential and basal cell fate commitment of mammary luminal progenitors: implications for basal-like breast cancer

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ABSTRACT

Mammary development requires a complex interaction between systemic hormones and locally produced growth factors. These molecules act through various receptors producing a signaling network that drives the correct growth, survival and organization of the developing mammary epithelium. Among the tyrosine kinase receptors that act downstream locally produced growth factors the IGFR, FGFR and ErbB family play a major role while little is known about the role of Met, the Hepatocyte Growth Factor Receptor.

Using mouse models, we found that mammary targeted Met knock-out driven by MMTV- and K14-cr transgene did not impair morphogenesis while pharmacological inhibition of Met indicated a reduction in the number of ducts. Checking for possible redundant mechanisms we found that inhibiting simultaneously Met and the EGF receptor, mammary ducts invasion of the fat pad was impaired leading to the formation of an immature mammary gland.

Examining the different mammary cell subpopulations we found that luminal progenitors express high levels of the Met receptor, as compared with the other mammary epithelial sub-populations. Constitutive activation of Met led luminal progenitors to attain stem cell properties, including enhanced clonogenic activity in vitro and de novo ability to reconstitute mammary glands in repopulation assays in vivo. Moreover, in response to Met signaling, luminal progenitors gave rise to hyperplastic ductal morphogenesis and preferentially underwent basal lineage commitment at the expense of luminal cell-fate specification. Opposite and symmetric results were produced by systemic pharmacological inhibition of Met.

Hence, Met signaling targets luminal progenitors for expansion, impairs their differentiation toward the mature luminal phenotype and enables their commitment toward the basal lineage.

Figure 1. Met signaling is not altered by genetic MMTV- or K14-driven mammary deletion, but is partially impaired by pharmacological inhibition of Met.

(A) Conditional deletion of exon 11 of mouse met-gene or whole mount images of met-deleted mammary glands using Cre recombinase driven under MMTV- or K14-cre promoters. (B) Representative whole mount images of outgrowths derived from transplantation of total mammary epithelial cells in mice treated with vehicle or with the Met inhibitor SU5402 (JNJ). Graph: average number of ducts/section. (KRT14 labeling in representative sections of ducts from untreated and JNJ treated outgrowths. Graph: average number of KRT14 positive cells.)

Figure 2. Met signaling is more severely impaired by concurrent inhibition of Met and EGFR.

Representative whole mount images of endogenous mammary glands stained on the Lynch nodal from mice treated with vehicle or the indicated compounds.

Figure 3. Activation of Met leads to hyperplasia and increased ductal branching of the mammary gland and enhances the frequency of luminal progenitors repopulating units.

(A) Representative whole mount images of outgrowths derived from mock and HGF transfected cells. Graph: average number of branches and bifurcations. (B) Frequency of mammary progenitors, units in repopulating outgrowths derived from total epithelial cells transfected with mock or HGF-expressing lentivector. (C) Hematoxylin and eosin (H&E) staining is representative sections of mock and HGF-expressing mammary outgrowths. Graph: average number of ducts/section. KRT14 labeling of ducts in representative sections from mock and HGF-expressing outgrowths. Graph: average number of KRT14 positive cell.

Figure 4. Met is preferentially expressed in ER luminal progenitors, where it stimulates clonogenic activity.

(A) Mammary epithelial lineages can be separated by flow cytometric analysis based on expression levels of CD24, CD117 and CD49f antigens. The CD24highCD49flowScal− subcluster contains myoepithelial cells and is enriched in basal cell sub-populations. Luminal estrogen receptor-positve (SER) cells have a CD24highCD49fhighScal− phenotype, and basal-like CD24lowCD49fhighScal+ cells are target ER-negative (ER−) luminal progenitors. (B) Representative images of luminal progenitors, epithelial cells and ER− luminal progenitors. Further fractionation of the basal sub-population to separate the CD24high fraction indicated the enrichment of luminal expression in the sub-set.

Conclusions:

We show that mammary targeted Met knock-out driven by MMTV- and K14-cr transgenes does not impair morphogenesis while pharmacological inhibition of Met reduces the number of ducts. Concomitant inhibition of Met and EGFR more severely impairs morphogenesis than inhibition of Met alone.

We provide evidence that Met is preferentially expressed in ER− luminal progenitors and that its activation by ex vivo gene transfer of HGF stimulates clonogenic activity in vitro, confers repopulating potential in vivo and promotes aberrant branch morphogenesis.

We also show that Met signaling restricts terminal differentiation of luminal progenitors and favors their commitment toward a more basal phenotype.

Finally we show that in a mouse model of BLBC, Met appears to be highly expressed in CD24+ER− cancer cells that resemble normal luminal progenitors.

Suggested readings:


