MET SIGNALING REGULATES GROWTH, REPOPULATING POTENTIAL AND BASAL CELL-FATE COMMITMENT OF MAMMARY LUMINAL PROGENITORS: IMPLICATIONS FOR BASAL-LIKE BREAST CANCER.

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

Paolo Accornero[1], Stefania Gastaldi[2], Francesco Sassi[3,4], Davide Torti[3,4], Silvia Miretti[1], Eugenio Martignani[1], Mario Baratta[1], Matthew J Smalley[5], Andrea Bertotti[3,4] and Livio Trusolino[3,4]

1 Department of Veterinary Morphophysiology, University of Torino School of Veterinary Medicine, Grugliasco, Italy; 2 Massachusetts General Hospital, Cutaneous Biology Research Center, Charlestown, MA 02129, USA; 3 Laboratory of Molecular Pharmacology, Institute for Cancer Research and Treatment (IRCC), Candiolo, Italy; 4 Department of Oncological Sciences, University of Torino School of Medicine, Candiolo, Italy; 5 The Breakthrough Breast Cancer Research Centre, The Institute of Cancer Research, London, UK

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-cre transgenes 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.

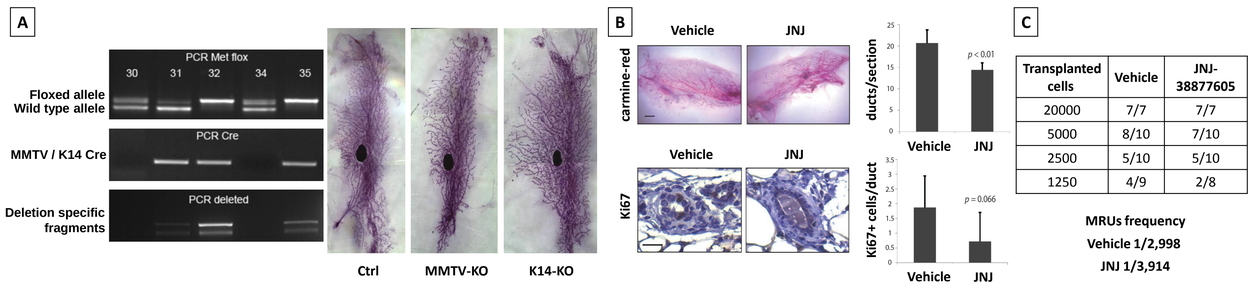

Figure 1. Mammary morphogenesis is not altered by genetic MMTV- or K14-driven mammary deletion, but is partially impaired by pharmacological inhibition of Met.
(A) Conditional deletion of exon 16 of mouse *c-met* gene and whole mount images of Met deleted mammary glands using Cre recombinase expressed under K14- or MMTV-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 JNJ-38877605 (JNJ). Graph: average number of ducts/section. Ki67 labeling in representative sections of ducts from vehicle-treated and JNJ-treated outgrowths. Graph: average number of Ki67-positive cells/duct.
(C) Frequency of mammary repopulating units in epithelial outgrowths derived from total epithelial cells in mice treated with vehicle or JNJ.

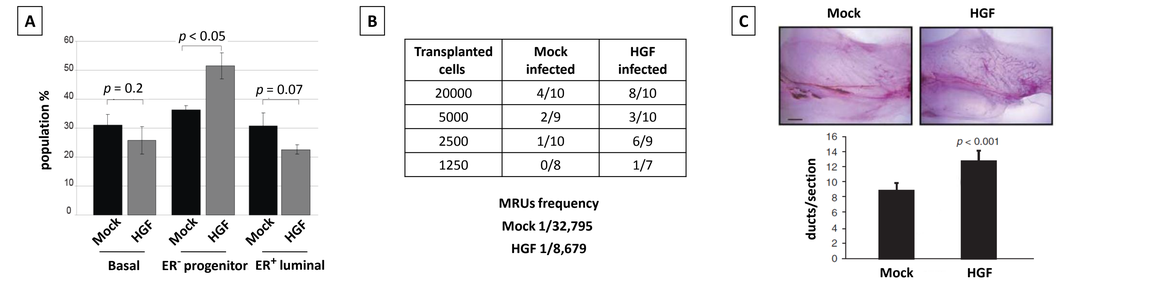

Figure 5: Met activation stimulates proliferation and mammary repopulating potential of luminal progenitors *in vivo*.
(A) Distribution of the mammary epithelial sub-populations in outgrowths derived from mock and HGF-expressing total mammary epithelial cells.
(B) Frequency of mammary repopulating units in epithelial outgrowths derived from ER$^-$ luminal progenitors transduced with mock or HGF-expressing lentiviral vectors.
(C) Representative whole mount images of the epithelial outgrowths derived from ER$^-$ luminal progenitors transduced with mock or HGF-expressing lentiviral vectors. Graph: average number of ducts/section.

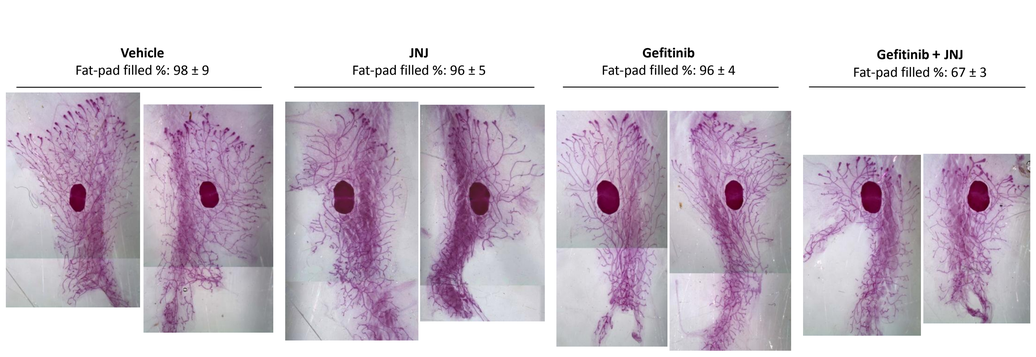

Figure 2. Mammary morphogenesis is more severely impaired by concurrent inhibition of Met and EGFR.
Representative whole mount images of endogenous mammary glands (aligned on the lymph node) explanted from mice treated with vehicle or the indicated compounds.

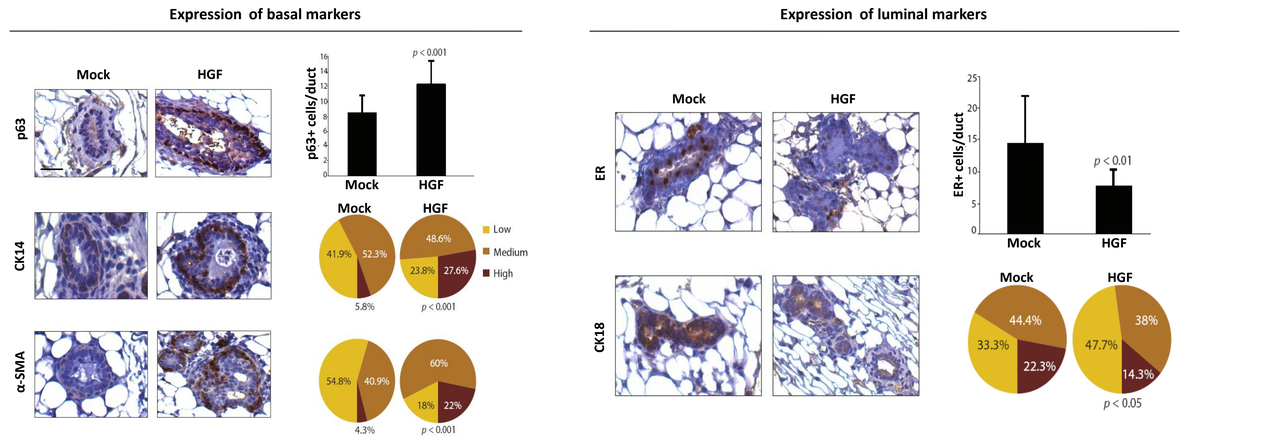

Figure 6: Constitutive activation of Met in luminal progenitors funnels cell fate toward a more basal phenotype.
Immunohistochemical staining for differentiation markers in representative sections of ducts from outgrowths derived from mock and HGF-expressing luminal progenitors. p63, CK14 and αSMA were selected as basal markers. ER and CK18 were selected as luminal markers. Graphs show the average number of p63 or ER positive cells/duct or percentage of cells with high/medium/low CK14, αSMA or CK18 expression.

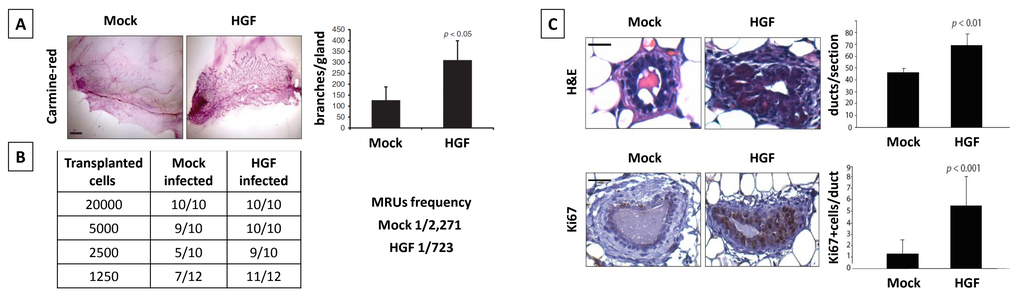

Figure 3. Activation of Met leads to hyperproliferation and increased ductal branching of the mammary gland and enhances the frequency of mammary repopulating units.
(A) Representative whole mount images of outgrowths derived from mock and HGF-transduced cells. Graph: average number of branches and bifurcations.
(B) Frequency of mammary repopulating units in epithelial outgrowths derived from total epithelial cells transduced with mock or HGF-expressing lentiviral vectors.
(C) Hematoxylin and eosine (H&E) staining in representative sections of mock and HGF-expressing mammary outgrowths. Graph: average number of ducts/section. Ki67 labeling of ducts in representative sections from mock and HGF-expressing outgrowths. Graph: average number of Ki67-positive cells/duct.

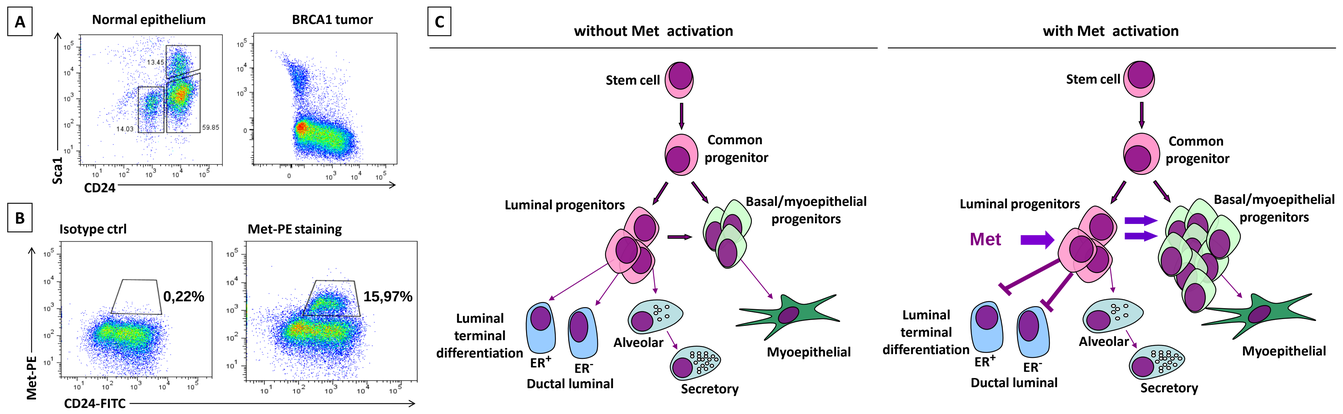

Figure 7: Met is highly expressed in tumors from a mouse model of basal-like breast cancer (Blg-Cre Brca1$^{f/f}$ p53$^{+/-}$).
A) FACS profile of a tumor from Blg-Cre Brca1$^{f/f}$ p53$^{+/-}$ mice. The three sub-populations cannot be discerned. The Sca1$^+$ fraction denoting differentiated, ER$^+$ luminal cells is virtually absent.
B) FACS staining of Met expression in the tumor.
C) Model depicting the mechanism by which Met activation restricts terminal differentiation of luminal progenitors and favors their commitment toward a more basal phenotype.

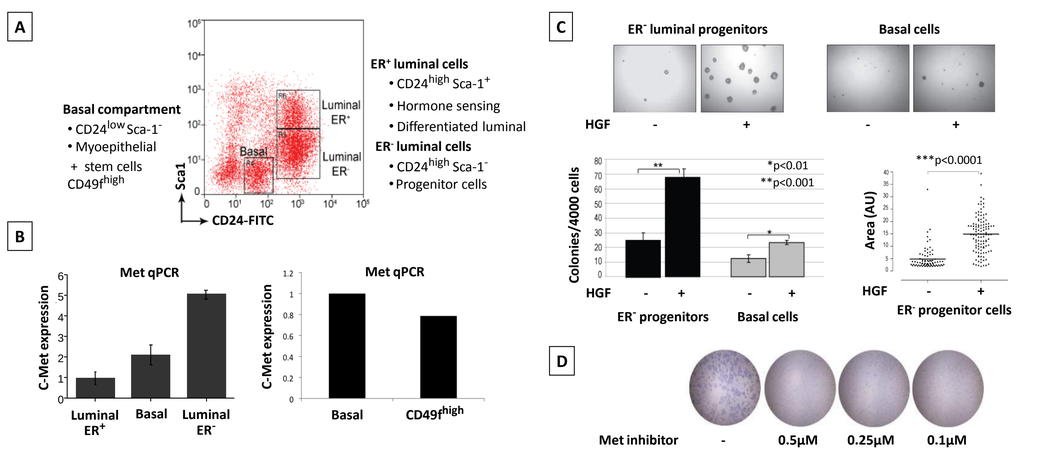

Figure 4. Met is preferentially expressed in ER$^-$ luminal progenitors, where it stimulates clonogenic activity.
(A) Mammary epithelial lineages can be separated by flow cytometric analysis based on expression levels of CD24, Sca1 and CD49f antigens. The CD24$^{+/low}$ Sca1$^-$ subset contains myoepithelial cells and is enriched in basal stem cells. Luminal estrogen receptor-positive (ER$^+$) cells have a CD24$^{+/high}$ Sca1$^+$ phenotype, and finally CD24$^{+/high}$ Sca1$^-$ cells are largely ER-negative (ER$^-$) luminal progenitors.
(B) qRT–PCR analysis of Met mRNA expression in freshly sorted sub-populations: differentiated ER$^+$ luminal cells, basal cells and ER$^-$ luminal progenitors. Further fractionation of the basal sub-population to separate the CD49f high subset indicated no enrichment of Met expression in stem cells.
(C) Clonogenic response to HGF in Matrigel cultures: representative images of sorted ER$^-$ luminal progenitors and basal cells with or without exogenous HGF. Histograms show quantitation of the number of colonies. Dot-plots of the average size of colonies in vehicle-treated or HGF-treated luminal progenitors and basal cells (arbitrary units).
(D) Two-dimensional colony formation assays: representative images of epithelial colonies formed by ER$^-$ luminal progenitors plated on a feeder layer of irradiated NIH-3T3 fibroblasts and treated with increasing doses of JNJ.

Conclusions:

We show that mammary targeted Met knock-out driven by MMTV- and K14-cre transgenes does not impair morphogenesis while pharmacological inhibition of Met reduces in 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 branching 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$^{+/high}$ cancer cells that resemble normal luminal progenitors.

Collectively, these findings shed new light onto the mechanisms that control morphogenetic events and cell-fate decisions in the mammary gland and have important implications for the role of Met in breast tumorigenesis.

Suggested readings:
Gastaldi S et al. Breast Cancer Res 2010; 12: 208.
Graveel CR et al. Proc Natl Acad Sci USA 2009; 106: 12909–12914.
Lim E et al. Nat Med 2009; 15: 907–913.
Molyneux G et al. Cell Stem Cell 2010; 7: 403–417.
Perou CM et al. Nature 2000; 406: 747–752.
Ponzo MG et al. Proc Natl Acad Sci USA 2009; 106:12903–12908.
Stingl J, et al. Nature 2006; 439: 993–997.
Trusolino L et al. Nat Rev Mol Cell Biol 2010; 11: 834–848.
Van Keymeulen A et al. Nature 2011; 479: 189–193.