BMP2, a key to uncover luminal breast cancer origin linked to pollutant effects on epithelial stem cells niche

Marion Chapellier¹ and Véronique Maguer-Satta²,3,4,5,*

¹Department of Clinical Genetics; Lund University; Lund, Sweden; ²CNRS UMR5286; Center de Recherche en Cancérologie de Lyon; Lyon, France; ³Inserm U1052; Center de Recherche en Cancérologie de Lyon; Lyon, France; ⁴Université de Lyon; Lyon, France; ⁵Department of Immunity, Virus and Microenvironment; Lyon, France

Keywords: bisphenol, BMP, BMPR1B, carcinogen, epithelial stem cell, breast tumor, niche, pollutant

Although current data implicate bone morphogenetic proteins (BMPs) in late stages of tumorigenesis and metastasis, we demonstrated that BMP2 also plays a role in promoting the transformation process of immature cells at very early stages.¹

Using a model of immature cells (MCF10-A cells), we have shown both in vitro and in vivo that chronic exposure of epithelial cells to BMP2 promotes their malignant transformation in an inflammatory context that is mimicked by interleukin 6 (IL6). Using an Affymetrix dataset together with transcript analysis of freshly isolated tumor cells, we established that BMP2/IL6-transformed cells display a luminal breast tumor-like phenotype. We identified bone morphogenetic protein receptor type 1B (BMPR1B) as the main BMP receptor expressed by transformed MCF10-A cells and by human luminal tumors. Although the role of BMPR1B in breast tumors remains unclear, we confirmed that it has a function specific to luminal tumors extending from the earliest stages up to established tumors and might constitute a potent biological marker of tumor progression. We identified small mothers against decapentaplegic 5 (SMAD5), GATA binding protein 3 (GATA3), and the forkhead box A1 and C1 (FOXA1/FOXC1) balance as potential BMP2 targets involved in the luminal epithelial cell transformation process. Our data suggest that upon binding of BMP2 to the BMPR1B receptor, epithelial stem cells upregulate SMAD5. Interestingly, BMP/SMAD5 signals negatively regulate the NODAL pathway that controls the expression of FOXH1, an activator of FOXC1 transcription.² SMAD5 could then replace SMAD1 in SMAD4 complexes, leading to repression of the FOXH1 promoter and a rapid decrease in FOXC1 transcription. Synergistically, SMAD5 complexes could directly induce GATA3 expression,³ also repressing FOXC1. Expression of estrogen-dependent genes would then commence and promote luminal epithelial cell commitment.

We showed that the BMPR1B ligands BMP2 and BMP4 are present in the normal mammary gland, where they function differentially and regulate stem/progenitor cell fate.⁴ Using primary human breast stem cells, we confirmed that only BMP2 expression is able to commit cells to differentiation and expansion of the luminal progenitor compartment, according to the role of BMPs in the development of the luminal lineage of murine mammary gland (summarized in Fig. 1 upper panel).⁴ Interestingly, we previously reported that, despite their strong homology and similar functions in mice, BMP2 and BMP4 regulate the human hematopoietic system differently, with BMP2 being involved in erythroid lineage and BMP4 in stem cell amplification and megakaryocyte lineage.⁵,⁶ Our data in both systems thus reveal that BMP2 seems to preferentially affect lineage-committed progenitors, whereas BMP4 has broader effects on stem cells and phenotypically related cells (megakaryocytic or myoepithelial progenitors). However, it is not known how these molecules distinctly signal in immature human cells to determine cell fate decisions.

The luminal progenitors compartment has been reported to have enhanced susceptibility to oncogenic events that might confer more genetically unstable features and increase BMP-specific targets such as BMPR1B to control stem cell fate.⁷ The evolution toward luminal tumors might
then be driven by specific microenvironmental disruption of available amounts of soluble BMP2. Binding of BMP2 to BMPR1B rapidly induces sustained signaling involving GATA3 and a change in the FOXA1/FOXC1 balance, leading to expansion of the luminal immature progenitors compartment that is further transformed through BMPR1B-dependent signaling. Therefore, it is possible that upon BMP2 signaling, transformation arises either from a stem/basal cell that first engages toward the luminal lineage before proliferation and further progress, or directly emerges from an already genetically altered committed luminal progenitor. Although based on our findings we cannot rule out either of these 2 hypotheses, we revealed the key active role that BMP2 can play in early phases of epithelial cell transformation.

Irrespective of the impact of BMP2 on luminal lineage commitment, analysis of normal and tumor tissue indicated that tumor cells themselves are the target rather than the origin of BMP2 overproduction. The transformation process may either lead to the loss of BMP2 expression in mammary epithelial cells or may occur in an epithelial cell that does not produce BMP2. We suspected that transforming agents that perturb signaling by niche components lead to local accumulation of BMP2 and IL6. We showed that radiation or environmental pollutants such as bisphenol A (BPA), a contaminant of food and drink through the use of plastic containers, and its substitute bisphenol S (BPS) are able to shift the balance of secreted BMP molecules in favor of BMP2. This seems to happen more frequently in individuals susceptible to...
developing breast cancer. Furthermore, IL6 secretion by stromal cells can be induced by BPA and BMP2, suggesting a possible feedback loop that maintains transforming conditions. Accordingly, the high BMP2 staining detected in tumor endothelial cells revealed increased angiogenesis that resulted in a continuous influx of BMP-laden platelets, thereby sustaining local high concentrations of BMPs. Indeed, human platelets are a main source of BMPs and could contribute to an early local increase in the concentration of soluble BMP molecules within the pretumoral niche. In addition, a local increase in BMP2, which is directly involved in breast microcalcification, could explain the clinical observation that microcalcification, one of the earliest mammographic signs, is associated early on with an increased risk of breast cancer. Taking all these observations into account, it is tempting to speculate that many other regulators or targets to ensure the same biological or oncogenic function to induce malignant transformation, our results illustrate the power of the stem cell niche to deliver exogenous cues that promote transformation and dictate the ultimate breast tumor type. Clinically, it might therefore be more efficient to develop new strategies that simultaneously and synergistically affect exogenous and endogenous target pathways rather than to concentrate on single drug targets.

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

Acknowledgments
We thank Sarah Kabani (CRCL) for excellent English proof reading assistance and Servier for providing images.

Funding
This study was funded by INSERM, Canceropole Rhône-Auvergne (CLARA), La Ligue Nationale Contre le Cancer (Ain, Rhône), ARC (SFI20111203500) and partly by ANR (ANR-10-LABX-0061 and 2011 ANR-CESA-018-04) and Region Rhône-Alpes (CMIRA-COOPERERA-12-004945-01, CMIRA-COOPERERA 2014 OTP431681), INCA grants to V.M.S. PhD-fellowships for M.C were from the French government and ARC.

References
1. Chapellier M, Bachelard-Cascales E, Schmidt X, Clément F, Treilleux I, Delay E, Jannot A, Méntrié-Caux C, Pochon G, Besançon R, et al. Disequilibrium of BMP2 Levels in the Breast Stem Cell Niche Launces Epithelial Transformation by Overamplifying BMPR1B Cell Response. Stem Cell Reports 2015; 4 (2):239-54; PMID:25601208; http://dx.doi.org/10.1016/j.stemcr.2015.01.007
2. Massague J, Xi Q. TGF-beta control of stem cell differentiation genes. FEBS Lett 2012; 586(14):1953-8; PMID:22219353; http://dx.doi.org/10.1016/j.febslet.2012.03.023
3. Bonilla-Claudio M, Wang J, Bai Y, Klysik E, Selever J, Gopalakrishnan R, Yee D, Graf D, Schwertfeger KL, Martin JF. Bmp signaling regulates a dose-dependent transcriptional program to control facial skeletal development. Development 2012; 139(4):709-19; PMID:22319775; http://dx.doi.org/10.1242/dev.073197
4. Forisman CL, Ng BC, Heinze RK, Kuo C, Sergi C, Gopalakrishnan R, Yee D, Graf D, Schwertfeger KL, Petryk A. BMP-binding protein twisted gastrulation is required in mammary gland epithelium for normal ductal elongation and myoepithelial compartmentalization. Dev Biol 2013; 373(1):95-106; PMID:23103586; http://dx.doi.org/10.1016/j.ydbio.2012.10.007
5. Jeanpierre S, Nicolini FE, Kaniewski B, Dumontet C, Rimokh R, Puisieux A, Maguer-Satta V. BMP4 regulation of human megakaryocytic differentiation is involved in thrombopoietin signaling. Blood 2008; 112 (8):3154-3163; PMID:18664625; http://dx.doi.org/10.1182/blood-2008-03-145326
6. Maguer-Satta V, Bartholin L, Jeanpierre S, Ffrench M, Martel S, Magaud JP, Rimokh R. Regulation of human erythropoiesis by activin A, BMP2, and BMP4, members of the TGFbeta family. Exp Cell Res 2003; 282 (2):110-20; PMID:12531607; http://dx.doi.org/10.1016/S0014-4827(02)00013-7
7. Molyneux G, Geyer FC, Magnay FA, Magnay FA, McCarthy A, Kuo C, Petryk A. BMP-binding protein twisted gastrulation is required in mammary gland epithelium for normal ductal elongation and myoepithelial compartmentalization. Dev Biol 2013; 373(1):95-106; PMID:23103586; http://dx.doi.org/10.1016/j.ydbio.2012.10.007
8. Ben Jonathan N, Hugo ER, Brandebourg TD. Effects of bisphenol A on adipokine release from human adipose tissue: Implications for the metabolic syndrome. MolCell Endocrinol 2009; 304(1-2):49-54; PMID:19433247; http://dx.doi.org/10.1016/j.mce.2009.02.022
9. Hyys SL, Olivares-Navarrete R, Hutton DL, Tan C, Boyas BD, Schwartz Z. Microstructured titanium regulates interleukin production by osteoblasts, an effect modulated by exogenous BMP-2. Acta Biomater 2013; 9(3):5821-9; PMID:23213301; http://dx.doi.org/10.1016/j.actbio.2012.10.030
10. Liu F, Bloch N, Bhushan KR, de Grand AM, Tanaka E, Solano S, Meryn PA, Goldberg N, Frangi JY, Lenkis RE. Humoral bone morphogenetic protein 2 is sufficient for inducing breast cancer microcalcification. MolImaging 2008; 7(4):175-86; PMID:19123988