Commentary

Stem Cell INVESTIGATION

Blocking TGF-β and BMP SMAD-dependent cell differentiation is a master key to expand all kinds of epithelial stem cells

Boris Guyot[1,2,3], **Véronique Maguer-Satta**[1,2,3]

[1]Université de Lyon, Université Claude Bernard Lyon 1, Lyon, F-69008, France; [2]Université de Lyon, Université Claude Bernard Lyon 1, INSERM U1052, CNRS 5286, Centre Léon Bérard, Centre de Recherche en Cancérologie de Lyon, Lyon, F-69008, France; [3]Department of Tumor Escape Signaling, Centre Léon Bérard, Lyon, France

Correspondence to: Boris Guyot. Cancer Research Center of Lyon-CRCL, U1052-UMR5286, 28 rue Laennec, 69373 Lyon Cedex 08, France. Email: boris.guyot@lyon.unicancer.fr; Véronique Maguer-Satta. Cancer Research Center of Lyon-CRCL, U1052-UMR5286, 28 rue Laennec, 69373 Lyon Cedex 08, France. Email: veronique.maguer-satta@lyon.unicancer.fr.

Provenance: This is an invited Commentary commissioned by Editor-in-Chief Zhizhuang Joe Zhao (Pathology Graduate Program, University of Oklahoma Health Sciences Center, Oklahoma City, USA).

Comment on: Mou H, Vinarsky V, Tata PR, *et al*. Dual SMAD Signaling Inhibition Enables Long-Term Expansion of Diverse Epithelial Basal Cells. Cell Stem Cell 2016;19:217-31.

Received: 18 November 2016; Accepted: 23 November 2016; Published: 08 December 2016.

doi: 10.21037/sci.2016.11.15

View this article at: http://dx.doi.org/10.21037/sci.2016.11.15

Epithelia are widely found in human tissues where they play various functions, providing mechanical protection, regulating exchanges between a body cavity and the underlying tissue or secreting various molecules inside or outside the body. This plethora of epithelia is susceptible to a large number of inherited or sporadic diseases impacting their functions. To cite but a few, cystic fibrosis is an inherited autosomal recessive disorder due to mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene coding for a chloride ion channel (1). CFTR loss in lung, pancreas, liver kidney and intestine results in epithelia malfunctions, respiratory complications being the most life-threatening symptom of cystic fibrosis. Inherited mutations in the Breast Cancer susceptibility (BRCA) 1 or 2 tumor suppressor genes predispose to breast and ovarian cancers originating from epithelial cells transformation (2). The ability to grow normal and pathological primary human adult epithelial cells in culture and to subject them to genetic manipulations is then of paramount importance for the fundamental studies of these diseases and for the development of efficient treatments.

Adult stem cells sustain many epithelia and often reside close to the basement membrane among the basal cells from where they can differentiate in mature epithelial cells. Co-culture strategies with irradiated fibroblasts allowing *in vitro* epithelial stem cells expansion have been developed several decades ago (3) and refinements using Rho-associated kinases (ROCK) inhibitors have been recently introduced (4). However, long-term expansion and manipulation of adult epithelial stem cells are still problematic and severely limit the ability to study patient samples.

Transforming growth factor β (TGF-β) and BMP signaling are widely involved in stem cells proliferation and differentiation in different types of epithelia such as breast (5) or skin (6). In particular, BMP4 has been involved in stem cell maintenance and in the control of the myoepithelial mammary lineage (5). On the other hand, it has been shown both at embryonic (7) and adult (5) stem cell level, that BMP2 specify the luminal differentiation of mammary epithelial progenitors by regulating GATA3 transcription factor. This exemplifies the various effects that different members of the BMP-TGFβ family can have on stem cells fate.

Specific antagonists of BMP and TGF-β have been previously used to improve epithelial organoid cultures (8,9). Rajagopal and colleagues recently attempted to improve epithelial basal cells long-term expansion by manipulating SMAD signaling (10). SMAD are intracellular molecules transducing TGF-β and bone morphogenetic proteins (BMP) signaling to the nucleus where they act as transcriptional regulators (11,12).

Using sections of mouse trachea and human bronchus

and immuno-fluorescence approaches, Rajagopal and colleagues looked at phospho-SMAD1/5/8 and phospho-SMAD2/3 which specifically mark BMP and TGF-β signaling, respectively. They showed that cytokeratin 8 (KRT8) positive mature luminal cells displayed high levels of both pSMAD1/5/8 and pSMAD2/3, revealing a dual activation of TGF-β and BMP pathways. Inversely, $p63^+$/$KRT5^+$ epithelial stem cells were largely negative for pSMAD1/5/8 and SMAD2/3. The few epithelial stem cells showing TGF-β/BMP activation were non-proliferating and displayed a weak KRT8 staining, suggesting early differentiating cells. These previous observations were extended to five other mouse epithelia derived from each of the three germ layers. In every case a strong association of dual TGF-β/BMP pathways activation with differentiated epithelial cells but not with proliferating epithelial stem cells was found.

These observations supported the hypothesis that TGF-β/BMP pathways played a role in diverse epithelia differentiation. This was tested by using two experimental settings allowing to follow mouse airway basal stem cells differentiation: mouse tracheal epithelia regeneration after sulfur dioxyde injury and human airway stem cell mucociliary differentiation in air-liquid interface culture (ALI). In both case, SMAD signaling was absent in basal stem cells but correlated with the appearance of differentiated luminal cells. Using genetic and pharmaceutical approaches, Rajagopal and colleagues then tested the requirement for BMP and TGF-β signaling in airway basal stem cells differentiation. $GSIb4^+$/$EpCAM^+$ tracheal basal stem cells from mice with BMPR1A (a BMP receptor), TGF-βRII (a subunit of the TGF-β receptor) and SMAD4 (a co-regulatory SMAD required for both BMP and TGF-β signaling) floxed alleles were transduced with Cre recombinase and differentiated in ALI cultures. This and similar approaches on human cells showed that the integrity of BMP and TGF-β pathways was required for normal luminal differentiation.

Since the authors showed that BMP and TGF-β pathways were highly activated in non-cycling, differentiating luminal cells, they tested the ability of inhibitors of these pathways to promote epithelial stem cell growth *in vitro*. Dual SMAD inhibition using both DMH-1 (a BMP pathway inhibitor) and A-83-01 (a TGF-β pathway inhibitor) allowed a feeder-free expansion of human and mouse airway stem cells. In these conditions, airway stem cells cultures reached 20 to 25 passages compared to the 6 passages attained without inhibitors. This amazing proliferation potential was associated with a decreased doubling time, a complete block of luminal differentiation and the uniform expression of stem cells markers. Very interestingly, early arrest of epithelial stem cells proliferation in absence of dual SMAD inhibition is not due to telomere attrition but rather to premature differentiation. On the other hand, the proliferation arrest in presence of dual SMAD inhibition seen after passage 25 is however due to telomere loss. Importantly, dual SMAD inhibition allowed the expansion of FACS-sorted single airway epithelial stem cells from normal donors and cystic fibrosis patients. In addition, human airway stem cells expansion was also successfully achieved from non-invasive procedures like bronchoalveolar lavage. Alterations in the TGF-β and BMP pathways are commonly found in cancers, but xenografts combined with transcriptomic and genome stability analysis convincingly showed that epithelial stem cells expansion following dual SMAD inhibition was not leading to tumorigenesis. Crucially, expanded airway stem cells kept their ability to differentiate in luminal cells even at late (20) passages when stem cells cultivated in absence of TGF-β/BMP pathways inhibition lost any differentiation ability at passage 5. These differentiating cells were able to form a mature and functional airway epithelium in ALI culture, as judged by histology and immunocytology as well as pharmaceutical studies on the CFTR ion channel.

Based on the initial observation than SMAD activation was seen in epithelial cells from all germ layers, the authors tested the dual SMAD inhibition conditions on a large panel of epithelial cells. A huge improvement in human keratinocytes cultures was achieved, since dual SMAD inhibition allowed a considerable expansion of human keratinocytes in feeder-free conditions, beyond passage 20, when in classical conditions the culture stops before passage 10 and is strictly dependent on the presence of feeder cells. Again, these expanded keratinocytes were able to form stratified epithelia very similar to human skin in ALI culture. Similar experiments on esophageal, epididymis and mammary epithelial cells showed that dual SMAD inhibition is a very general and versatile mean of expanding functional epithelial stem cells and progenitors able to form proper epithelia complying with their tissue of origin.

This study should constitute an important breakthrough in the field. The ability to expand functional epithelial stem cells from a variety of tissues both from human patients and mouse models of human diseases could open new experimental avenues so far constrained by technical limitations inherent to classical culture protocols. Notably,

the capacity of the dual SMAD inhibition culture to allow the cloning of human epithelial stem cells obtained from minimally invasive procedures could allow the development of genetically modified human, and even patient specific, cells models. As stated by the authors, further improvements of culture conditions are however needed for the expanded stem cells to keep their full potential to differentiate into physiologically normal epithelia at later passages.

TGF-β and BMP pathways have been described as either tumor suppressors or promoters depending on the context (13,14). This duality has been largely documented for TGF-β which is antitumoral at an early stage but pro-tumoral at latter stages. Although BMP signaling role in metastasis is still unclear (15,16), we recently showed that it was involved in early phases of breast cancer. Indeed, chronic long-term exposure to high levels of soluble BMP2 could initiate the luminal transformation of pre-disposed breast epithelial stem cells through binding to BMPR1b (5). In addition, environmental pollutants such as estrogeno-mimetic bisphenols, are able to initiate deregulations of the BMP signaling by altering BMP pathway members expression within epithelial stem cells, as well as BMP expression itself by their microenvironment (5,17). In this context, it is interesting to observe an absence of induced tumorigenesis after long-term dual TGF-β and BMP pathways inhibition in primary epithelial stem cells.

This work highlights the crucial roles of TGF-β and BMP in epithelial stem cells. Deciphering the regulation of TGF-β and BMP production by the microenvironment, their interplay with the several antagonists modulating their interactions with their receptors and their downstream signaling and transcriptional networks will be an important step in understanding the control of epithelial stem cell fate.

Acknowledgements

The authors thank the Canceropôle Rhône-Auvergne (CLARA); ANR (ANR-10-LABX-0061 and 2011-ANR-CESA-018-04); Région Rhône-Alpes (CMIRA-COOPERA-12-004945-01 and CMIRA-COOPERA-14-007020); Foundations: La Ligue Nationale Contre le Cancer (Ain, Rhône and Saône-et-Loire), ARC (SFI20111203500) and Déchaîne Ton Coeur for their support.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Mall MA, Hartl D. CFTR: cystic fibrosis and beyond. Eur Respir J 2014;44:1042-54.
2. O'Donovan PJ, Livingston DM. BRCA1 and BRCA2: breast/ovarian cancer susceptibility gene products and participants in DNA double-strand break repair. Carcinogenesis 2010;31:961-7.
3. Rheinwald JG, Green H. Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. Cell 1975;6:331-43.
4. Liu X, Ory V, Chapman S, et al. ROCK inhibitor and feeder cells induce the conditional reprogramming of epithelial cells. Am J Pathol 2012;180:599-607.
5. Chapellier M, Bachelard-Cascales E, Schmidt X, et al. Disequilibrium of BMP2 levels in the breast stem cell niche launches epithelial transformation by overamplifying BMPR1B cell response. Stem Cell Reports 2015;4:239-54.
6. Oshimori N, Fuchs E. Paracrine TGF-β signaling counterbalances BMP-mediated repression in hair follicle stem cell activation. Cell Stem Cell 2012;10:63-75.
7. Forsman CL, Ng BC, Heinze RK, et al. BMP-binding protein twisted gastrulation is required in mammary gland epithelium for normal ductal elongation and myoepithelial compartmentalization. Dev Biol 2013;373:95-106.
8. Sato T, Vries RG, Snippert HJ, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 2009;459:262-5.
9. Tadokoro T, Gao X, Hong CC, et al. BMP signaling and cellular dynamics during regeneration of airway epithelium from basal progenitors. Development 2016;143:764-73.
10. Mou H, Vinarsky V, Tata PR, et al. Dual SMAD Signaling Inhibition Enables Long-Term Expansion of Diverse Epithelial Basal Cells. Cell Stem Cell 2016;19:217-31.
11. Hata A, Chen YG. TGF-β Signaling from Receptors to Smads. Cold Spring Harb Perspect Biol 2016;8.
12. Miyazono K, Kamiya Y, Morikawa M. Bone morphogenetic protein receptors and signal transduction. J Biochem 2010;147:35-51.
13. Ehata S, Yokoyama Y, Takahashi K, et al. Bi-directional roles of bone morphogenetic proteins in cancer: another molecular Jekyll and Hyde? Pathol Int 2013;63:287-96.
14. Principe DR, Doll JA, Bauer J, et al. TGF-β: duality of function between tumor prevention and carcinogenesis. J

Natl Cancer Inst 2014;106:djt369.
15. Buijs JT, van der Horst G, van den Hoogen C, et al. The BMP2/7 heterodimer inhibits the human breast cancer stem cell subpopulation and bone metastases formation. Oncogene 2012;31:2164-74.
16. Owens P, Pickup MW, Novitskiy SV, et al. Inhibition of BMP signaling suppresses metastasis in mammary cancer. Oncogene 2015;34:2437-49.
17. Clément F, Xu X, Donini CF, et al. Long-term exposure to bisphenol A or benzo(a)pyrene alters the fate of human mammary epithelial stem cells in response to BMP2 and BMP4, by pre-activating BMP signaling. Cell Death Differ 2016. [Epub ahead of print].

doi: 10.21037/sci.2016.11.15
Cite this article as: Guyot B, Maguer-Satta V. Blocking TGF-β and BMP SMAD-dependent cell differentiation is a master key to expand all kinds of epithelial stem cells. Stem Cell Investig 2016;3:88.