RESEARCH HIGHLIGHT

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Prevention of breast cancer by RANKL/RANK blockade

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Robust genetic evidence in mice and humans indicates that RANK signaling plays a major role in mammary carcinogenesis driven by *BRCA1*/*BRCA2* mutations. These findings may inaugurate a new era of breast cancer prevention, changing the life of millions of women worldwide.

According to current estimates, one in eight women will suffer from breast cancer during her lifetime. Several factors have been associated with an increased risk for breast cancer development, including post-menopausal hormonotherapy, oral contraceptives, obesity and genetic predisposition [1]. Indeed, as much as 2%-10% of breast cancer cases are associated with germline mutations in *BRCA1* and *BRCA2*, which encode two proteins with a central role in the repair of DNA double-strand breaks. The current standard of care for women who have a familial history of breast cancer and carry *BRCA1* or *BRCA2* mutations is bilateral radical mastectomy. Such a surgical procedure, which has recently been under the limelight owing to the Angelina Jolie case, has complex psychological repercussions and is not 100% effective [1]. Moreover, the implementation of population-wide mammography-based screening campaigns failed to decrease the incidence of breast neoplasms that are metastatic at presentation, and might *per se* increase the risk of breast cancer development [2]. This implies that mammary carcinogenesis does not proceed according to the model originally proposed by William Stewart Halsted (who hypothesized that tumors form at a single location, grow there and eventually disseminate) [2], calling for the development of novel prophylactic tools. Recent work from Josef Penninger's group provides robust genetic and pharmacological evidence in support of the notion that breast cancer developing in the context of *BRCA1* or *BRCA2* mutations can be prevented by blocking RANKL/RANK signaling [3]. Since a RANKL-targeting monoclonal antibody (i.e., denosumab) is currently approved by the US Food and Drug Administration (FDA) and equivalent agencies worldwide for the treatment of multiple bone conditions and has an exceptional safety record [4, 5], these findings may pave the way to a new era of breast cancer prophylaxis, changing the life of millions of women worldwide.

Based on previous findings from their group demonstrating an essential role for RANK in mammary gland development and progestin-driven mammary carcinogenesis [6, 7], Penninger and collaborators initially set out to determine the impact of RANK signaling in multiple genetic models of mammary carcinogenesis, including: (1) mice bearing homozygous $Brca1^{fl/fl}$ alleles in a $Trp53^{fl/fl}$ context (targeting the master oncosuppressor p53) and expressing the Cre recombinase under the control of the *keratin 5* (*Krt5*) promoter, which is active in multiple epithelia; and (2) $Brca1^{fl/fl}Trp53^{fl/fl}$ mice expressing the Cre^{C} recombinase under the control of the *whey acidic protein* (*Wap*) promoter, which is specifically active in luminal and basal mammary epithelial cells independently of doxycycline and pregnancy. To this aim, they crossed the strains described here above with $Tnfrsf11a^{fl/fl}$ mice (in which the RANK-coding sequence is floxed), and monitored not only tumor incidence over time, but also biochemical and pathological parameters of developing neoplasms, including markers of DNA damage, proliferation rate and grade [3]. Tumors developing in the absence of RANK manifested similar degrees of DNA damage and proliferation rate as tumors developing in a RANK-proficient background. However, the deletion of *Tnfrsf11a* not only led to reduced tumor grade in both models, but also delayed tumor onset in the $WapCre^{C}$ model (which can be maintained for long periods, at odds with the *Krt5Cre* model that succumbs to skin tumors at around 4 months of age). Moreover, whereas all mice lacking *Brca1* and *Trp53* in mammary progenitor cells (as per $WapCre^{C}$-dependent recombination) developed breast neoplasms by approximately 7 months of age, ~ 25% of mice lacking *Brca1*, *Trp53* and *Tnfrsf11a* never developed breast neoplasms [3].

To confirm their observations in a clinically relevant model, Penninger and colleagues took advantage of $Brca1^{fl/fl}$ mice expressing Cre under the control of the *mouse mammary tumor virus* (*MMTV*) promoter, which is also preferentially active in the breast epithelium. In this setting, mammary carcinogenesis is driven solely by the absence of *Brca1*, which closely mimics the situation of women with germline *BRCA1* or *BRCA2* mutations. By the age of 9 months, 33% of these mice spontaneously developed pre-neoplastic lesions as a result of *Brca1* loss, which was completely abrogated by the subcutaneous administration of a RANK-targeting antibody fragment. At 15 months of age, as many as 82% mice maintained in control conditions (i.e., receiving an irrelevant antibody fragment) manifested pre-neoplastic breast lesions, while only 7% of mice receiving the RANK-targeting

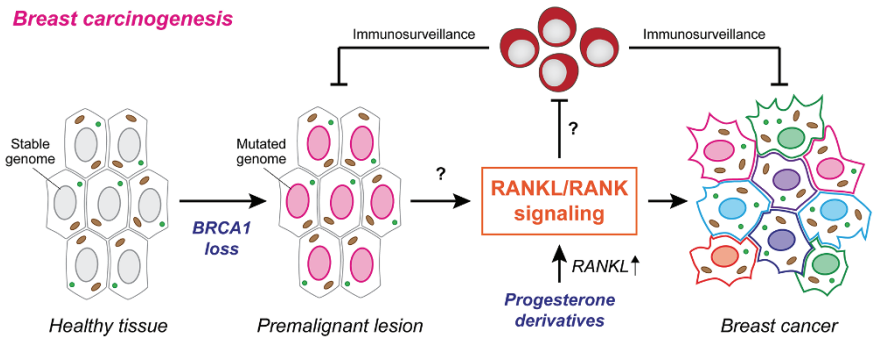

Figure 1 RANKL/RANK signaling in breast cancer. Mammary carcinogenesis driven by *BRCA1* mutations relies on autocrine or paracrine RANKL/RANK signaling in mammary progenitor cells. Breast cancers developing as a consequence of *BRCA1* mutations also depend on progesterone signaling (knowing that progesterone derivatives also promote mammary carcinogenesis), most likely as a result of progesterone receptor-driven RANKL expression and consequent proliferation of mammary progenitor cells. The mechanisms linking the accumulation of genetic defects to carcinogenesis via the RANKL/RANK system, as well as the possible impact of RANKL/RANK signaling in the mammary epithelium on anticancer immunosurveillance remain to be determined.

agent did so [3]. These findings demonstrate that blocking RANKL/RANK limits mammary carcinogenesis driven by *BRCA1* mutations, at least in mice.

RANK was also involved in the expansion of murine $Lin^{-}CD24^{+}CD49f^{hi}$ basal mammary progenitor cells in a model of ovariectomy followed by sham treatment or estrogen plus progesterone administration. To confirm the validity of their findings in the human system, Penninger and collaborators isolated mammary progenitor cells from 3 women with germline *BRCA1* mutations who underwent prophylactic mastectomy and tested their sensitivity to denosumab in clonogenic assays. Human *BRCA1*-deficient progenitor cells treated with denosumab had a reduced clonogenic potential as compared to the same cells kept in control conditions [3]. These data pointed to the involvement of RANK in human mammary carcinogenesis driven by *BRCA1* mutations, lending additional support to the possibility that the blockade of RANKL/RANK may constitute a clinically implementable strategy. To corroborate even further this hypothesis, Penninger and colleagues undertook a multipronged analysis of the RANKL/RANK system in clinical settings to draw two major conclusions. First, RANK and RANKL are expressed at high levels only by breast cancers with *BRCA1* or *BRCA2* mutations, and RANK protein levels exhibit an exquisite correlation with tumor grade in this scenario (in a clinical cohort of ~250 breast cancer patients). Second, common *TNFRSF11A* polymorphisms that increase RANK expression levels are associated with an increased risk for breast cancer development in women with *BRCA1* or *BRCA2* mutations (in a large cohort from the Collaborative Oncological Gene-environment Study cumulatively including 23 000 women with breast cancer) [3].

Of note, the ability of *BRCA1* mutations to drive mammary transformation has previously been shown to rely on proficient progesterone signaling [8], which is known to drive RANKL expression in the mammary epithelium [7, 9]. It will therefore be interesting to fully characterize the molecular mechanisms through which the progesterone system and RANKL/RANK cooperate to support carcinogenesis driven by *BRCA1* or *BRCA2* mutations. Moreover, it will be important to evaluate whether and how RANK affects natural or therapy-induced immunosurveillance against breast cancer [10, 11] (Figure 1). Irrespectively, the findings by Penninger and co-authors may inaugurate a new era in which women with *BRCA1* or *BRCA2* mutations receive denosumab as an efficient prophylaxis instead of undergoing bilateral radical mastectomy. It remains to be seen whether such a prophylactic treatment would also reduce the incidence of ovarian carcinoma driven by *BRCA1* or *BRCA2* mutations.

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References

1 Bianchini G, Balko JM, Mayer IA, *et al. Nat Rev Clin Oncol* 2016 May 17. doi:10.1038/nrclinonc.2016.66
2 Welch HG, Gorski DH, Albertsen PC. *N Engl J Med* 2015; **373**:1685-1687.
3 Sigl V, Owusu-Boaitey K, Joshi PA, *et al. Cell Res* 2016; **26**:761-774.
4 Lacey DL, Boyle WJ, Simonet WS, *et al. Nat Rev Drug Discov* 2012; **11**:401-419.
5 Vacchelli E, Aranda F, Eggermont A, *et al. Oncoimmunology* 2014; **3**:e27048.
6 Fata JE, Kong YY, Li J, *et al. Cell* 2000; **103**:41-50.
7 Schramek D, Leibbrandt A, Sigl V, *et al. Nature* 2010; **468**:98-102.
8 Poole AJ, Li Y, Kim Y, *et al. Science* 2006; **314**:1467-1470.
9 Tanos T, Sflomos G, Echeverria PC, *et al. Sci Transl Med* 2013; **5**:182ra155.
10 Kroemer G, Senovilla L, Galluzzi L, *et al. Nat Med* 2015; **21**:1128-1138.
11 Galluzzi L, Buque A, Kepp O, *et al. Cancer Cell* 2015; **28**:690-714.