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RANK/RANKL Axis in Melanoma

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1. Introduction

The TNF receptor superfamily member 11A known as receptor activator of nuclear factor κB (RANK/TNFRSF11A), its ligand RANKL (TNFSF11) and the decoy receptor for RANKL called osteoprotegerin (OPG/TNFRSF11B) have been shown to be key regulators of bone remodeling (Simonet et al., 1997; Lacey et al., 1998; Theoleyre et al., 2004). Indeed, RANKL mediates osteoclastogenesis and activates mature osteoclasts, whereas OPG negatively regulates RANKL binding to RANK, reduces the half-life of membranous RANKL and finally inhibits bone resorption by osteoclasts (Tat et al., 2006). Together with such part in bone, the RANK/RANKL axis is also involved in a variety of physiologic functions. Certainly, the RANK/RANKL axis controls the lymph-node organogenesis, the thymic medullary epithelial cells differentiation, the central thermoregulation, the formation of lactating mammary gland during pregnancy and the proliferation of epithelial cells of the epidermo-pilosebaceous unit (Dougall et al., 1999; Kong et al., 1999; Fata et al., 2000; Rossi et al., 2007; Hanada et al., 2009; Duheron et al., 2011).
In parallel to its physiologic functions, the RANK/RANKL axis has been also implicated in several pathologies, in particular in tumors with bone connections as bone primitive tumors and bone metastasis forming tumors. Thus, functional RANK expression has been reported in cells of different tumors, such as prostate and breast cancers, osteosarcoma and melanoma (Jones et al., 2006; Wittrant et al., 2006; Mori et al. 2007a, b, c). Moreover, RANKL was shown to trigger the migration of these RANK-expressing cells (Jones et al., 2006; Mori et al., 2007a). According to these observations, the RANK/RANKL axis might have a great impact on melanoma development. The aim of the present chapter is to discuss, based on the actual knowledge, the feasibility of targeting RANK/RANKL axis for the treatment of melanoma.

2. RANK/RANKL axis interest for melanoma treatment

2.1 RANK/RANKL, skin-appendages and skin

The RANK/RANKL axis has emerged as an important physiologic player in epithelial cell growth and differentiation. First evidences came from expression patterns of both RANK

and RANKL during the development of skin-appendages as hairs, teeth and mammary glands (Ohazama et al., 2004; Mikkola, 2008; Tanos & Brisken, 2008; Duheron et al., 2011). Regarding mammary glands, RANK and its ligand are both expressed in epithelial cells and control the development of a lactating mammary gland during pregnancy. In absence of RANK/RANKL signaling, the formation of lobulo-alveolar structures, necessary to a functional lactating mammary gland, is severely impaired leading to milk secretion defect.

Concerning hairs, RANK is expressed by the hair follicle germ, bulge stem cells and epidermal basal cells. Interestingly, these cell-types are implicated in the renewal of the epidermo-pilosebaceous unit. Its ligand (RANKL) is actively transcribed by the hair follicle at initiation of its growth phase, providing a mechanism for RANK-expressing stem cell engagement and hair-cycle entry. Mice deficient in RANKL are unable to initiate a new growth phase of the hair cycle and display arrested epidermal homeostasis. Furthermore, transgenic mice overexpressing RANK in the hair follicle or administration of recombinant RANKL both activate the hair cycle and epidermal growth. Finally, RANK signaling is dispensable for the formation of the stem cell compartment and the induction of hair follicle mesenchyme, but RANK-RANKL axis regulates hair renewal and epidermal homeostasis and provides a link between these two activities.

The RANK/RANKL axis also plays essential roles on immune system including participation in T-cell/dendritic cell communications (Leibbrandt & Penninger, 2010). Interestingly, RANKL over-expression in keratinocytes results in functional alterations of epidermal dendritic cells and systemic increases of regulatory CD4(+)CD25(+) T cells. Consequently, epidermal RANKL expression can modify dendritic cell functions to maintain the number of peripheral CD4(+)CD25(+) regulatory T cells. Finally, environmental stimuli at the skin level can rewire the local and systemic immune system by means of RANKL.

2.2 RANK/RANKL and bone-associated cancers

Since the late nineteenth century, it has been thought that the microenvironment of the local host tissue actively participates in the tendency of some cancers to metastasize to specific organs (Paget, 1889). However, the specific factors involved are still unknown.

Bones are continuously remodeled throughout life by two complementary processes: bone matrix formation (apposition) regulated by osteoblasts and bone resorption managed by osteoclasts. The precise inter-relation between osteoblasts and osteoclasts leading to osteoclastogenesis is only partly deciphered. The discovery of certain key factors involved in the control of osteoclastogenesis has moved bone research into a new era. Current findings have revealed that the RANKL/RANK/OPG molecular triad constitutes a key regulator for both normal and pathological bone metabolism (Brown et al., 2001; Goltzman, 2001; Chen, et al. 2006). The prevention of different tumors metastases inheritance in bone by RANKL inhibitors [*i.e.*, OPG or soluble RANK (sRANK) or RANK blocking antibodies (RANK-Fc)] in established animal models of bone metastases, highlights the critical role of this triad in cancer-induced bone manifestations (Zhang et al., 2001, 2003; Corey et al., 2005; Whang et al., 2005; Mountzios et al., 2007; Canon et al, 2008). Interestingly, such anti-tumor effects appear to be restricted to bone models. Indeed, such effects have not been observed in any other models, including classical subcutaneous models (Zhang et al., 2001, 2003). Thus, it

was first believed that anti-tumor effects induced by RANKL/RANK interaction blockage were the result of an indirect effect *via* osteoclasts.

In turn, functional RANK expression was recently reported in bone-associated tumors, more precisely in cells of breast cancer, prostate cancer, osteosarcoma and malignant melanoma (Jones et al., 2006; Wittrant et al., 2006; Mori et al., 2007a, b, c; Armstrong et al., 2008). According to the fact that RANK-expressing tumor cells migration was induced by RANKL stimulation (Armstrong et al., 2008; Jones et al., 2006; Mori et al., 2007a), the direct effect of RANKL on RANK-expressing tumor cells was in fine disclosed. Consequently, RANKL works, in bone, as one of the "soil" factors of Paget's theory (Fig. 1).

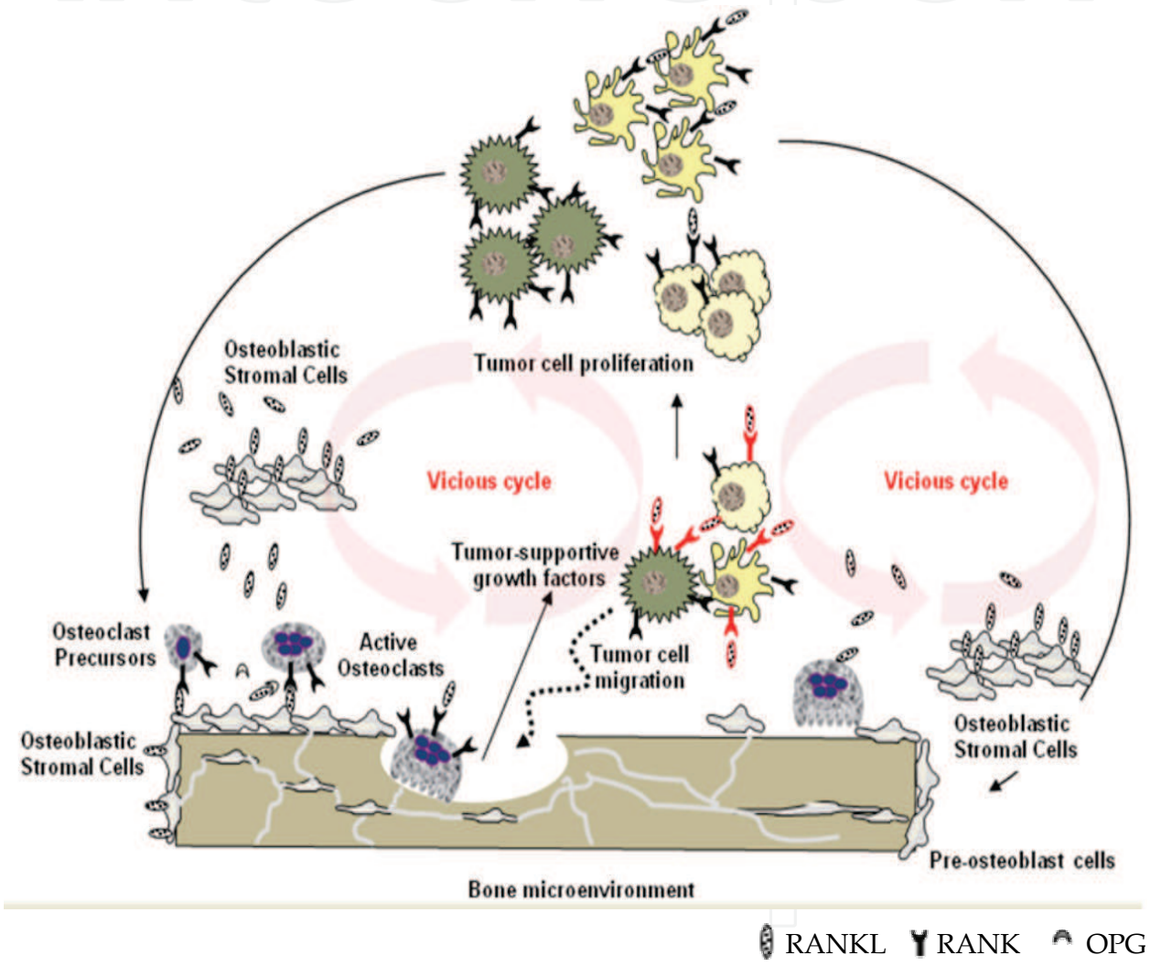

Fig. 1. Schematic representation of the putative interactions between RANK-expressing tumor cells (*i.e.,* prostate cancer, breast cancer and malignant melanoma) and bone cells (osteoblasts and osteoclasts) in the tumoral bone microenvironment

In fact, RANK-expressing tumor cells would preferentially targeted bone microenvironment where RANKL concentration is elevated. In the bone tumoral environment, RANKL produced by osteoblasts and bone stromal cells has two potential targets: on the one hand the osteoclast precursor and the osteoclast, and on the other hand the RANK-expressing

tumor cell. So RANKL acts as a "soil" factor that facilitates cancer metastasis settlement in bone by activating both kinds of RANK-expressing cells.

2.3 Melanomas: skin tumors with bone metastasis

Melanoma belongs to the large family of skin tumors (see WHO classification of skin tumors: In Pathology and Genetics of Skin Tumors edited by P.E. LeBoit, G. Burg, D. Weedon and A. Sarasin, IARC Press, Lyon, 2006). From a clinical and public health point of view, malignant melanomas are the most important group of skin tumors. Although less common than basal and squamous cell tumors of the skin, they are much more often fatal, due to their intrinsic propensity to metastasis. The major environmental risk factor for melanoma is recurrent expositions to high-doses of UV radiations. Endogenous factors are often combined as genetic susceptibility. Bone metastasis is a poor prognostic for patient and corresponds to the ultimate stage of the pathology. The precise implication of RANK/RANKL axis in the bone metastatic process has been controversial but nowadays it seems clear that this signalization plays successive parts in this complex process. Indeed, RANK/RANKL axis is implicated in tumor cell migration (as previously described) and later in tumor cell settlement in the bone microenvironment and induction of osteolysis (Mundy, 2002; Jones et al., 2006). Consequently, targeting RANK/RANKL signalization might be a promising strategy to prevent melanoma bone metastasis and subsequent damages.

2.4 Targeting RANK/RANKL axis for melanoma treatment: benefit/risk

According to the disastrous consequences of melanoma metastasis in term of patient survival, any treatments that enable confinement of tumor cells to their initial site has to be considered as therapeutically beneficial. RANK/RANKL inhibitors, due to the implication of RANK/RANKL axis in metastatic process, are so potentially highly relevant therapeutic agents for melanoma. They may reduce the incidence of metastasis and synergized with anti-tumoral drugs. Indeed, several studies has been reported such beneficial effect of OPG (Lamoureux et al., 2007), OPG peptide (Heymann et al., 2005), RANK-Fc (Lamoureux et al., 2008) and Denosumab (fully human anti-RANKL antibody) (Abrahamsen et al., 2005) as RANK/RANKL inhibitors for the treatment of bone-associated cancers.

However, as presented above, the RANK/RANKL signalization is implicated in various physiological processes during development and takes part to the immune response. So targeting this pathway in children may have developmental consequences that need to be evaluated. Moreover, whatever the age of the patient, the potential impact of such inhibitor on the immune response as to be taken into account and may in fine limited their use.

3. Conclusion

The use of drugs targeting the RANK/RANKL axis in melanoma appears to be a promising strategy to reduce the mortality of this skin cancer. Such drugs should reduce the metastatic process and enforce the action of classical anti-tumoral treatment. However, further studies will be necessary to evaluate the impact of these drugs on RANK/RANKL signaling physiological functions, more specifically during growth, and to deal with these drugs potential wrong impact on immune system.

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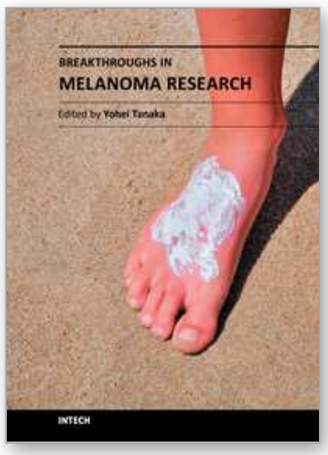

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Melanoma is considered to be one of the most aggressive forms of skin neoplasms. Despite aggressive researches towards finding treatments, no effective therapy exists to inhibit the metastatic spread of malignant melanoma. The 5-year survival rate of metastatic melanoma is still significantly low, and there has been an earnest need to develop more effective therapies with greater anti-melanoma activity. Through the accomplishment of over 100 distinguished and respected researchers from 19 different countries, this book covers a wide range of aspects from various standpoints and issues related to melanoma. These include the biology of melanoma, pigmentations, pathways, receptors and diagnosis, and the latest treatments and therapies to make potential new therapies. Not only will this be beneficial for readers, but it will also contribute to scientists making further breakthroughs in melanoma research.

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