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Chapter

Role of RUNX2 in Melanoma: A New Player in Tumor Progression and Resistance to Therapy

Rachael Pulica, Karine Cohen Solal and Ahmed Lasfar

Abstract

RUNX2, a transcription factor, initially known for its indispensable role in skeletal development. RUNX2 is essential for osteoblast differentiation and the maintain of the osteocyte balance. RUNX2 acts directly on osteoblasts via Fgf pathway or on mesenchymal progenitors through Hedgehog, Wnt, Pthlh and DLX5. Currently, many reports point its critical role in the progression and metastasis of several cancer types. RUNX2 is involved in EMT process, invasion and metastasis through the modulation of important oncogenic pathways, including Wnt, FAK/PTK and AKT. In melanoma, RUNX2 is a key player in mediating intrinsic RTK-associated pro-oncogenic properties. We have showed a dramatic up regulation of RUNX2 expression with concomitant up-regulation of EGFR, IGF-1R and AXL, in melanoma cells rendered resistant to BRAF mutant inhibitors. Approximately half of melanomas carry BRAF mutations which enhance tumor invasion and metastasis. In this chapter, we describe the potential mechanisms, leading to the upregulation of RUNX2 in melanoma with BRAF mutations. We also highlight the critical role of PI3K/AKT in the expression and activation of RUNX2, and its consequences on the regulation of many critical factors, controlling cancer invasion and metastasis.

Keywords: Cancer and metastasis, melanoma, RUNX2, BRAF, PI3K/AKT, Wnt, Pthlh and DLX5, EGFR, IGF-1R and AXL

1. Introduction

Runt-related transcription factor 2 (Runx2) belongs to RUNX family, consisting of three members, Runx1, Runx2, and Runx3. All members are highly conserved with a 128 amino acid DNA binding/protein binding domain runt. In contrast to other RUNX members, RUNX2 holds a variable poly-glutamine, poly-alanine repeat domain [1]. Natural discrepancy within this repeat could alter the transactivation potential of RUNX2 which acts as an evolutionary 'tuning button' for the control of suggested to skull shape. The role of Runx2 is critical in skeletal development, and its alteration or low expression often lead to skeletal dysplasia. Runx2 plays important role in the process of mesenchymal stem cells differentiation into osteoblasts, and ultimately to osteocyte. Runx2 is required for the proliferation of pre-osteoblasts in whole skeletons and mesenchymal cells in sutures. Indeed, Runx2 is required for the commitment of mesenchymal cells to osteoblast lineage cells [2]. Thus, Runx2 makes a condensed cell layer of uncommitted mesenchymal cells or osteoblast progenitors by increasing their proliferation and facilitates their differentiation into osteoblast

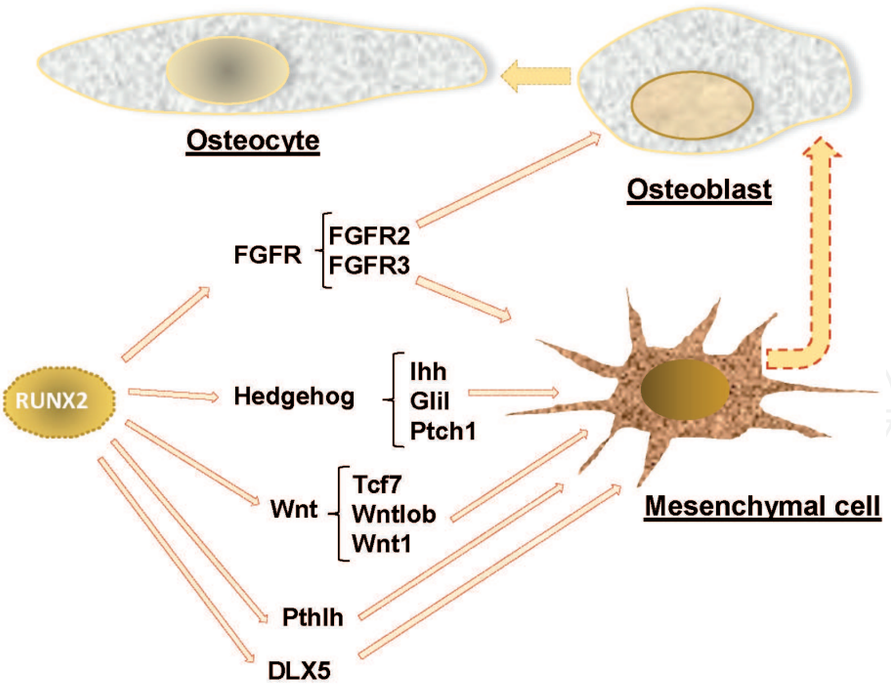

Figure 1.
RUNX2 and skeletal development. RUNX2 promotes osteoblast differentiation via several signaling pathways. RUNX2 directly acts on osteoblasts via Fgfr1 and Fgr2 to induce their differentiation and their promotion to osteocytes. RUNX2 acts on mesenchymal cells and induces specific pathways to enable osteoblast differentiation.

lineage cells. RUNX2 modulates the balance between osteoblasts and osteocytes, by either stimulating or inhibiting the osteoblast differentiation, occurring via the modulation of many factors and signaling pathways, including hedgehog signaling (Gli1, Ptch1 and Ihh), FGFR signaling (FGFR2 and FGFR3), Wnt signaling (Tcf7 and Wnt10b), Pth1r, Dlx5, Tnc, and Ncam1 (**Figure 1**). Defects or alterations in the expression or the activity of these factors or signaling pathways, may lead to skeletal dysplasia. Therefore, Runx2 could be used as target for the development of novel therapeutic strategies for bone-related diseases.

Besides, it is critical role in osteoblast differentiation, RUNX2 is also involved in the regulation of chondrocyte proliferation during bone formation. However, Runx2 expression in terminal hypertrophic chondrocytes is not essential for vascular invasion into the cartilage, but is for their survival and trans-differentiation into osteoblasts. Studies in animal models, showed that the trans-differentiation is required for trabecular bone formation in embryonic and neonatal stages, but not for procuring normal bone structure and volume in young and elder animals [3].

2. Multifaceted role of RUNX2 in cancer

The role of RUNX2 in cancer promotion has been well described in many cancer types. The common feature of those cancers is the elevated level of RUNX2 expression. Although, numerous similarities have been reported for the pro oncogenic role of RUNX2, some differences are also described (**Figure 2**).

In breast cancer (BC), early studies have shown a correlation between RUNX2 expression and the "Triple Negative" phenotype [4]. Analysis of tissue microarrays shown that high level of RUNX2 expression is associated with the triple-negative breast cancer phenotype and a low survival of BC patients, in comparison with patients, displaying reduced level of RUNX2 expression. Apparently, in triple negative cells, RUNX2 promotes Wnt and TGF-beta signaling [5]. RUNX2 is capable

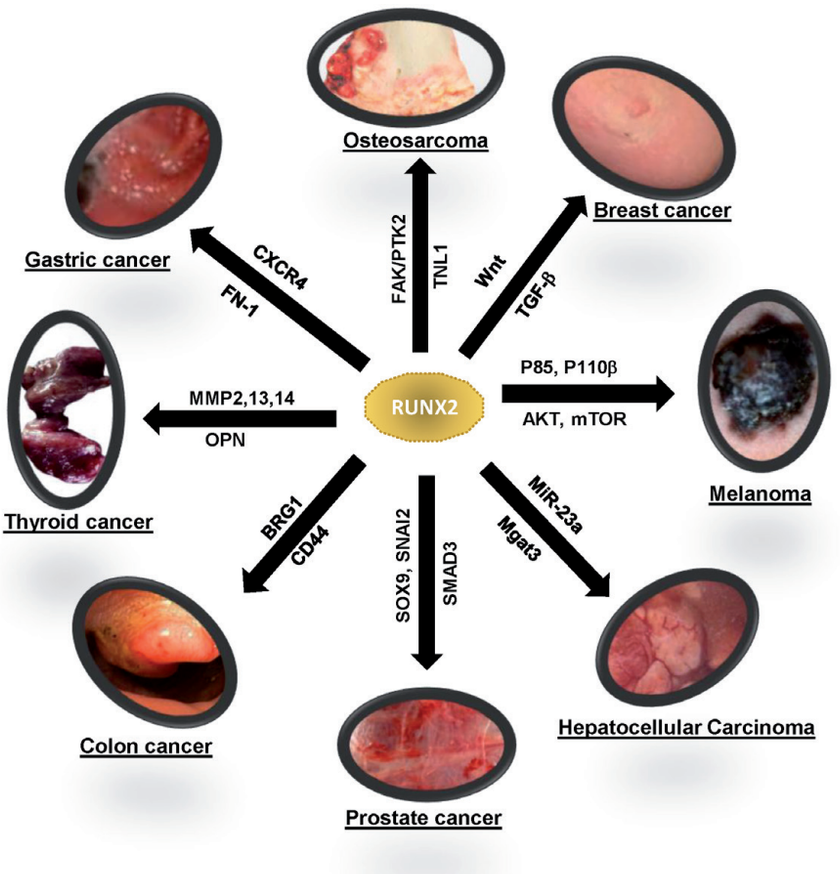

Figure 2.
RUNX2 and promotion of cancer. RUNX2 promotes cancer development of several cancer types: Melanoma, hepatocellular carcinoma, prostate cancer, colon cancer, thyroid cancer, gastric cancer, osteosarcoma and breast cancer. To promote cancer progression, RUNX2 induces several pathways, relevant to each cancer type.

to regulate different factors, playing critical role in either inhibiting or stimulating Wnt pathway. RUNX2 can interact with SMAD3 to promote TGF-beta signaling, in addition, to its direct interaction with the Estrogen receptor-alpha (ER-α), enabling the expression of aromatase, an estrogen producing enzyme. Increasing the level of estrogens, which in turn stimulate cell proliferation of BC cells [5].

It has been also reported that RUNX2 directly regulates TGFβ-induced levels of PTHrP, the level of MMP13 and MMP9, IL-8, bone sialoprotein and OPN [6–11]. Furthermore, we and others found that irregularities in RUNX2 expression induce EMT changes in some mammary epithelial cell lines and twists normal acini structure [6, 11, 12], strongly suggesting that RUNX2 plays a critical role in early breast cancer progression [6].

More recently, it has been demonstrated that RUNX2 was involved in breast cancer bone metastasis. This pro-metastatic role is mediated through integrin alpha5 [13].

In hepatocellular carcinoma (HCC), a significant increase of RUNX2 has been established in both HCC samples and cell lines. It has been demonstrated that RUNX2 promotes HCC cell migration and invasion via MMP9 [14]. In addition, RUNX2 increases the pro-metastatic process via MiR-23a and Mgat3 direct targeting [15].

In prostate cancer, RUNX2 has been also reported as cancer promotor. When RUNX2 is overexpressed in a C4-2B prostate cancer cell line, the invasiveness is greatly enhanced, and transcription factors involved in EMT (SOX9, SNAI2, and SMAD3) are upregulated [6, 16]. RUNX2 siRNA treatment of the prostate and breast cancer cells decreased migration and invasion of the cancerous cells [6, 17].

In gastric cancer (GC), a correlation between RUNX2 expression and invasion/metastasis has been established. Patients with GC tumors displaying low RUNX2 expression had a better outcome than those with high RUNX2 expression [18, 19]. RUNX2 was identified as an independent prognostic indicator for GC patients with a COX regression analysis. In an orthopedic GC nude mouse model, RUNX2 significantly increased the invasion and metastatic potential of the GC cells. *In vitro* studies reflected a significant increase in migration and invasion abilities of GC cells connected to an increase in RUNX2.

RUNX2 promotes metastasis and invasiveness of GC cells, via the chemokine receptor CXCR4 [18]. RUNX2 directly binds to the promotor region of CXCR4, enhancing its transcription and leading to overexpression in human GC cells. Knockdown of RUNX2 in GC cell lines results in a significant downregulation of CXCR4 mRNA. Additionally, CXCR4 is found to have a role in early-stage GC development by recruiting stromal cells and establishing a progenitor niche that favors tumor growth and development. However, it has been recently demonstrated that RUNX2 can negatively regulate the expression of Febronectin1 (FN1) [19], an important gene, playing critical role in tumor invasiveness and metastasis of GC [20, 21].

The role of RUNX2 has been also described in colon cancer. It has been found that RUNX2 promoted cell proliferation and invasion of colon cancer cells via estrogen/ERbeta pathway [10]. More recently, it has been demonstrated that RUNX2 could interact with BRG1 to target CD44 for promoting invasion and migration of colorectal cancer cells [22]. It has been also recently reported that Integrative multi-omics analysis of a colon cancer cells with heterogeneous Wnt activity reveals RUNX2 as an epigenetic regulator of Epithelial–mesenchymal transition (EMT), the critical process which promotes cancer metastasis, stemness and resistance to treatment [23].

The contribution of RUNX2 to the promotion of other cancer types, including thyroid cancer, osteosarcoma and melanoma has been also reported. RUNX2 activates expression of MMP2, MMP13, MMP14, and OPN, promoting the invasive and migratory activity of thyroid cancer cells [6, 24]. Osteosarcoma cells with siRNA depletion of RUNX2 show a reduction in motility. The genomic promoter of RUNX2 in osteosarcoma shows genes involved in cancer cell motility including FAK/PTK2 and TNL1 [6, 25]. The role of RUNx2 has been well studied in melanoma. Our group has extensively contributed to the understanding of the role of RUNX2 in this leading skin cancer.

3. Role of RUNX2 in melanoma promotion

Melanoma malignancy has a very high mortality rate and a resistance to chemotherapy [26]. Of these melanoma malignancies, a study reflected almost half of patients had bone metastases [27]. Melanocytes arise from the neural crest and show progressive stemness features. This renders melanoma to be such a highly metastatic cancer once the process has started [13]. Malignant melanoma has been described to have a higher expression level of RUNX2 than normal melanocytes [26]. As other cancer types, Runx2 has been investigated in connection to the progression, development, and metastasis of tumors as well as the epithelial to mesenchymal transition (EMT). It has been shown that the RUNT domain of RUNX2 affects EMT and promote bone metastasis in melanoma via several mechanisms, including WWTR1 and TGF-beta [26, 27].

The interaction of RUNX2 with the PI3K/AKT signaling pathway is critical for tumor invasion and metastasis [28]. AKT interacts with RUNX2 via different

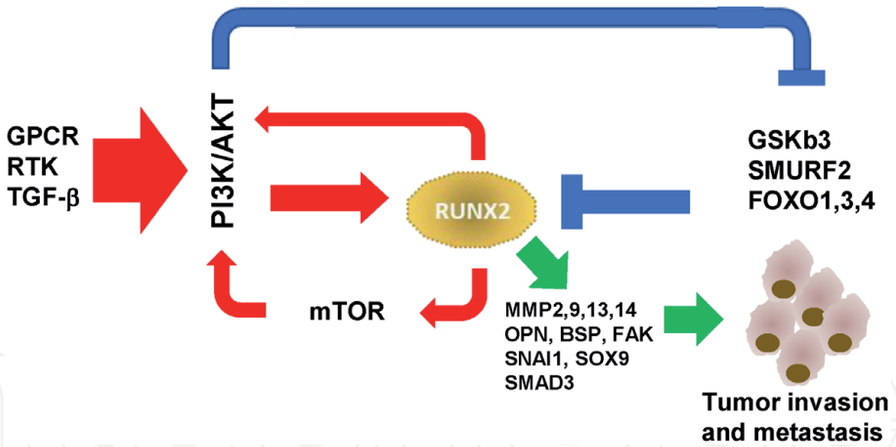

Figure 3.
Role RUNX2/PIT3K/AKT axis in cancer invasion and metastasis. PIT3/AKT promotes cancer invasion and metastasis via RUNX2. Activation loop between RUNX2 and PIT3/AKT enables the amplification of oncogenic signaling via many factors. Activation of RUNX2 lead to the promotion of cancer invasion and metastasis via the induction of MMPs and other factors.

mechanisms, including phosphorylating/activating of RUNX2 or RUNX2 modulators. Reciprocally, the activation of the PI3K/AKT pathway by RUNX2 has been also reported. This mutual activation, maintain a constitutive AKT activation and high expression of RUNX2 in cancer cells, and constitute one of a major driving force for tumor progression and metastasis in melanoma (**Figure 3**).

4. Role of RUNX2 in melanoma progression and acquired resistance to BRAFi

RUNX2 was initially described as one of the transcription factors whose expression was significantly correlated with elevated levels of the non-canonical signaling member of the WNT family, WNT5A, following chronic treatment (over 10 weeks) with the BRAF inhibitors PLX4720 and PLX4032 [29]. We previously showed that RUNX2-deficient melanoma cells, displayed a significant down-regulation of leading receptor tyrosine kinases, EGFR, IGF-1R, PDGFRβ and AXL. Our finding strongly suggested a critical role for RUNX2 in mediating intrinsic RTK-associated pro-oncogenic properties in melanoma. In addition, we demonstrated a significant up-regulation of RUNX2 expression and concomitant up-regulation of EGFR, IGF-1R and AXL in melanoma cells rendered resistant to PLX4720 [30]. We then reported that PLX4720-resistant cells developed in an *in vivo* context exhibit an increase in RUNX2 levels when re-exposed to PLX4720 *in vitro*. These findings strongly suggest that RUNX2 could play a critical role in acquired resistance to PLX4720. In order to address the relevance of these findings in human melanoma, clinical data from a cohort containing samples from untreated tumors and tumors treated with vemurafenib and dabrafenib respectively [31] were analyzed. Probes for all three main RUNX2 transcripts were represented on the array. We found that the expression of RUNX2 isoform 3 is significantly higher in vemurafenib-treated patients compared to the untreated group ($p = 0.0024$). These results showing the up-regulation of RUNX2 in melanoma lesions from patients treated with vemurafenib, strongly suggest that chronic exposure to BRAFi (PLX4720/vemurafenib) could favor RUNX2 up-regulation, leading to RTK up-regulation and the induction of acquired drug resistance to BRAFi [30].

The mechanism(s) leading to RUNX2 up-regulation in BRAFi-resistant melanoma cells have yet to be discovered. One possible mechanism would involve

WNT5A and the WNT5A-mediated activation of the PI3K/AKT pathway [29]. As RUNX2 expression is increased by the PI3K/AKT pathway signaling [28, 30], elevated WNT5A expression and subsequent AKT pathway activation could result in RUNX2 overexpression. Therefore, any kinase rewiring that leads to hyper-activated PI3K/AKT signaling in melanomas resistant to BRAFi [32] would provide a favorable context for high RUNX2 expression.

5. Conclusion

Besides, its indispensable role in bone development, the transcription factor RUNX2 is a critical player in the promotion of several cancers. Important oncogenic pathways, including PI3K/AKT axis are involved in mediating the effects of RUNX2. We believe that targeting RUNX2 or its modulators may open novel therapeutic avenues for cancer.

Acknowledgements

The authors thank all present and past laboratory members for their great contributions in progressing our understanding of RUNX2. American Cancer Society and NIH for funding.

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