



## Minireview

# The Role of RUNX1 in NF1-Related Tumors and Blood Disorders

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**Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder, NF1 patients are predisposed to formation of several type solid tumors as well as to juvenile myelomonocytic leukemia. Loss of NF1 results in dysregulation of MAPK, PI3K and other signaling cascades, to promote cell proliferation and to inhibit cell apoptosis. The RUNX1 gene is associated with stem cell function in many tissues, and plays a key role in the fate of stem cells. Aberrant RUNX1 expression leads to context-dependent tumor development, in which RUNX1 may serve as a tumor suppressor or an oncogene in specific tissue contexts. The co-occurrence of mutation of NF1 and RUNX1 is detected rarely in several cancers and signaling downstream of RAS-MAPK can alter RUNX1 function. Whether aberrant RUNX1 expression contributes to NF1-related tumorigenesis is not fully understood. This review focuses on the role of RUNX1 in NF1-related tumors and blood disorders, and in sporadic cancers.**

**Keywords:** cancer, mutation, neurofibromatosis type 1, RUNX1, tumor driver

## INTRODUCTION

Neurofibromatosis type 1 (NF1) is a common inherited human disorder, with a frequency of 1:2,500-1:3,500 worldwide (Boyd et al., 2009). NF1 encodes neurofibromin, a RAS

GTPase-activating protein (RAS-GAP) which inactivates RAS-GTP by accelerating the hydrolysis of RAS-GTP to RAS-GDP. In the absence of NF1, a series of signaling cascades including mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K) and other pathways are enhanced, resulting in the promotion of cell proliferation and inhibited apoptosis (Downward, 2003; Ratner and Miller, 2015). In NF1 patients, this can result in optic pathway glioma, glioblastoma, peripheral nerve neurofibromas, pheochromocytoma, aggressive nerve sarcoma (malignant peripheral nerve sheath tumor, MPNST), and/or juvenile myelomonocytic leukemia (JMML) (Ratner and Miller, 2015; Varan et al., 2016). Loss of NF1 is also common in sporadic tumor formation in glioma, breast cancer, lung cancer, and other cancers (Philpott et al., 2017).

The Runt-related transcription factor (RUNX) family of human and mouse genes includes RUNX1, RUNX2, and RUNX3 in both human and mouse. These gene products share many structural similarities but play distinct biological roles. RUNX1 (also called AML1, CBFA2) is required for maturation of megakaryocytes and differentiation of T and B cells (Sood et al., 2017). RUNX2 is critical for skeletal morphogenesis (Komori, 2010). RUNX3 is important for neurogenesis of proprioceptive neurons in the dorsal root ganglia (DRG) and for hematopoiesis (Levanon et al., 2002; Stifani and Ma, 2009). All three RUNX proteins bind to the core-binding factor beta (CBFB) via the same protein motif. CBFB lacks a DNA-binding domain, but binding to RUNX substantially increases the

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C/EBPβ RUNT domain DNA-binding affinity and protein stability, thereby enhancing RUNX transcriptional activities (Bushweller, 2000; Huang et al., 2001). Abnormal RUNX expression promotes the development of cancer via transcriptional misregulation, DNA repair defects, and genomic instability (Ito et al., 2015). Because RUNX1 is a sequence-specific DNA binding transcription factor, whether it functions as an oncogene or a tumor suppressor is dependent on its gain or loss of function, and/or on its interaction with specific co-regulatory proteins. RUNX1 has been implicated as a tumor suppressor in solid tumors including breast cancer, esophageal adenocarcinoma, colon cancer and possibly prostate cancer and as an oncogene in skin cancer, endometrial cancer, and epithelial cancer (Ito et al., 2015). RUNX2 has been implicated in metastasis, including bone metastasis (Akech et al., 2010). RUNX3 acts as a tumor suppressor in gastric cancer but functions as an oncogene in ovarian cancers (Ito et al., 2015). RUNX1 can be phosphorylated by ERK signaling (downstream of NF1/RAS/MAPK) at S276/S293 or be methylated by PMRT at R206 and R210 (Imai et al., 2004; Zhao et al., 2008). Our recent study showed that elevated ERK phosphorylation caused by loss of Nf1 increases Runx1 expression and contributes to neurofibroma formation (Li et al., 2016). Both NF1 and RUNX1 are drivers of tumor formation in several cancers. They might also coordinately contribute to tumor formation in some tumors. This review focuses on the role of RUNX1 in NF1-related tumors and blood disorders, and in sporadic cancers.

## RUNX1 IN NF1-RELATED SOLID TUMORS

### Neurofibromas

One of the hallmarks of NF1 is that about 95% of NF1 patients develop dermal neurofibromas, a benign Schwann cell tumor. About half of patients develop plexiform neurofibromas and about 10% of patients develop MPNSTs (Evans et al., 2011; Ratner and Miller, 2015; Varan et al., 2016). The molecular mechanisms of tumorigenesis and the molecules that drive neurofibroma formation are not fully understood. Schwann cells are the primary pathogenic cells in neurofibromas (Chen et al., 2014). They are the only cells in neurofibroma with biallelic NF1 mutation (Serra et al., 1997). Ablation of Nf1 using Nf1 homozygous (Nf1<sup>-/-</sup>) embryonic stem cells to make transgenic mice or ablation of Nf1 in the Schwann cell lineage using Krox20Cre or inducible PLPCre in mice leads to the development of plexiform neurofibromas (Chen et al., 2014; Cichowski et al., 1999; Mayes et al., 2011; Zhu et al., 2002). Loss of Nf1 in Schwann cells and Schwann cell precursors at embryonic day 12.5 (E12.5) in mice leads to plexiform and dermal neurofibromas (Wu et al., 2008). Dermal and plexiform neurofibromas also develop in mice using HOXB7-Cre or Prss56-Cre as a driver to ablate Nf1 in boundary cap cells (Chen et al., 2019; Radomska et al., 2019). Dermal neurofibromas develop from skin-derived precursors through loss of Nf1 (Le et al., 2009). Proliferation of Schwann cells that have lost most contact with nerve axons is a feature of neurofibroma formation (Zheng et al., 2008). These reports demonstrate that Schwann cell precursors and more mature Schwann cells can serve as cells of origin in neurofibromas.

RUNX1 is associated with stem cell function in many tissues and is a key regulator in the fate of stem cells, including hematopoietic stem cells, hairy follicle stem cells, mammary epithelial stem cells, mesenchymal stem cells, neural stem cells, and muscle stem cells (Deltcheva and Nimmo, 2017). RUNX1 plays an important role during the development in both the central and peripheral nervous systems. In the central nervous system, Runx1 promotes adult mouse neurosphere cell proliferation and neuronal differentiation (Logan et al., 2015). In the peripheral nervous system, Runx1 orchestrates gene expression changes during the differentiation and maturation of DRG neurons (Yoshikawa et al., 2013). Our recent studies show that Runx1 can also act as an oncogene to drive neurofibroma formation upon loss of Nf1 (Li et al., 2016). Unlike the RUNX1 chromosomal translocations and mutations frequently detected in other cancers, RUNX1 is overexpressed in human neurofibroma initiating cells, and in human plexiform neurofibromas, as well as in mouse Schwann cell precursors and mouse plexiform neurofibromas, at the mRNA and protein levels. Genetic or pharmacological inhibition of Runx1 function decreased mouse neurofibroma sphere growth in vitro, a measure of tumor-forming potential [16]. Consistent with reports that Runx1 promotes proliferation and neuronal differentiation in adult brain neurosphere culture, loss of Nf1 increased number of E12.5 Runx1<sup>+</sup>/FABP7<sup>+</sup> Schwann cell precursors that can enable tumor formation (Li et al., 2016). Dual genetic deletion of mouse Runx1 and Runx3 in Schwann cells and Schwann cell precursors significantly but incompletely delayed neurofibromagenesis and prolonged mouse survival. Elevated ERK signaling that is due to loss of Nf1 phosphorylates Runx1. This results in regulating peripheral myelin protein 22 (PMP22) expression in part by alternative promote usage, and inducing elevated levels of protein expression of PMP22, contributing to the effect on neurofibromagenesis (Hall et al., 2019). Of note, other signaling pathways such as Wnt, Notch, or Trp53-p21 can directly or indirectly activate Runx1, and might also contribute to neurofibroma initiation and/or maintenance (Li et al., 2016) (Fig. 1).

### Glioblastoma multiforme

The tumor suppressor gene NF1 is the third most prevalently mutated gene in human glioblastoma multiforme (GBM) in the general population. NF1 germline or somatic mutation is a driver of gliomagenesis (McLendon et al., 2008). In NF1 patients the risk of high grade glioma is 2- to 5-fold higher than in the general population (Spyris et al., 2019; Varan et al., 2016). Recently, RUNX1 was suggested to be another potential driver of mesenchymal GBM (Cooper et al., 2012; Zhao et al., 2019) (Fig. 2). RUNX1 serves as a master regulator of gene expression in the U87 GBM cell line, which shows low NF1 expression (Way et al., 2017). Augmented expression of RUNX1 deregulates global gene expression in the U87 GBM cell lines and inhibits tumor growth in mice (Bogoch et al., 2017). Teng et al. (2016) show that a histocompatibility leukocyte antigen (HLA) complex P5 (HCP5)-microRNA-139-RUNX1 feedback loop plays a pivotal role in regulating the malignant behavior of U87 and U251 glioma cells. However, elevated p38 MAPK signaling by IL-1β induced RUNX1

expression and increased the expression of the invasion- and angiogenic-related molecules that contribute to glioma metastasis and angiogenesis in U87 cells (Sangpairoj et al., 2017). Co-mutation of NF1 and RUNX1 might be rare, because analysis of the cancer genome atlas (TCGA) GBM data identified only 1 patient with both NF1 and RUNX1 mutations among 396 GBM patients with available mutation data (Kwangmin Choi, personal communication).

### Breast cancer

Patients with NF1 shows increased risk of developing breast cancer (Howell et al., 2017; Suarez-Kelly et al., 2019) (Fig. 2). A recent study shows that breast cancer was diagnosed in 32 of 404 NF1 patients (Uusitalo et al., 2017). Mutation of *Nf1* can also drive breast cancer in a mouse model (Wallace et al., 2012). Recent studies show that RUNX1 is also a driver

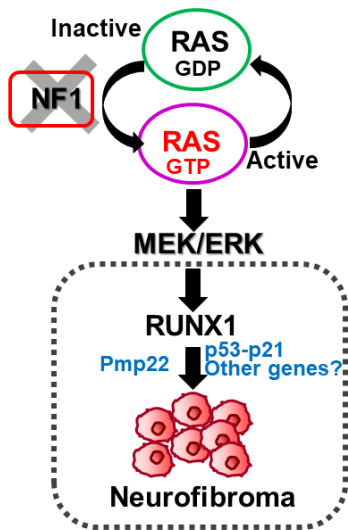
for breast cancer (Banerji et al., 2012). Whole genome and whole exome sequencing studies identified point mutations and deletions of RUNX1 in luminal and basal breast cancers (Banerji et al., 2012; Ellis et al., 2012; Hong et al., 2018; Rooney et al., 2017). RUNX1 is frequently mutated, as are other well-known tumor suppressors and oncogenes (including PTEN, CDH1, TP53, and PIK3CA) which have been extensively investigated in breast cancer. However, in the publicly available data from TCGA, there was also a low prevalence of NF1 and RUNX1 co-mutation/deletion (Desmedt et al., 2016). There were only 2 patients with both NF1 and RUNX1 mutations among 1066 breast cancer patients with mutation data from TCGA (Kwangmin Choi, personal communication), suggesting that NF1 and RUNX1 mutation might mutually exclusive in breast cancer.

### Lung cancer

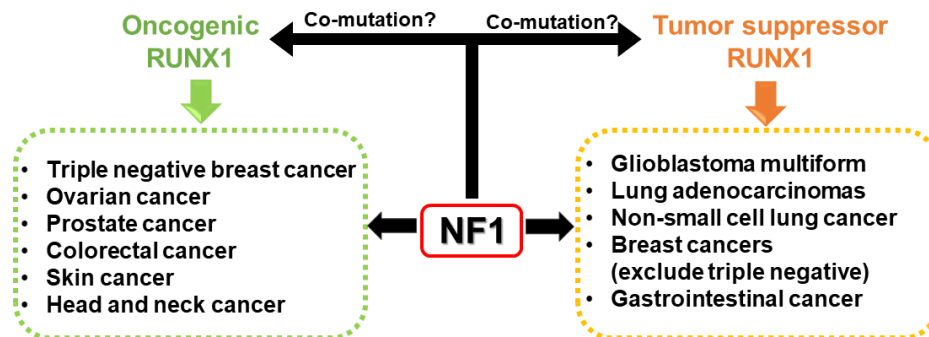
NF1 is also a significantly mutated gene in lung adenocarcinoma (Ding et al., 2008). NF1 is frequently mutated in a distinct molecular and clinical subtype of lung adenocarcinoma (Tlemsani et al., 2019). Contrary to its oncogenic function in neurofibroma, loss of RUNX1 is associated with aggressive lung adenocarcinomas (Ramsey et al., 2018), suggesting that RUNX1 serves as a tumor suppressor in lung cancer (Fig. 2). Low RUNX1 levels in lung adenocarcinomas were associated with worse overall survival. Loss of RUNX1 might drive lung adenocarcinoma aggression through deregulation of the E2F1 pathway (Ramsey et al., 2018). However, it is not known whether co-mutation/deletion of RUNX1 and NF1 will worsen the phenotype.

### Other solid cancers

RUNX1 or NF1 mutation predisposes to development of many other cancer types (Fig. 2). NF1 is the fourth most prevalently mutated gene in ovarian carcinoma (Bell et al., 2011), in which RUNX1 serves as an oncogene, contributing to cell proliferation, migration and invasion (Keita et al., 2013). NF1 mutations may be a critical progression gene in other cancers such as melanomas (Philpott et al., 2017). On very rare occasions, co-mutation of RUNX1 and NF1 is reported in post-transplant lymphoproliferative disorders patient exophytic tumor in the small bowel (Bogusz, 2017).



**Fig. 1. Loss of NF1 activates RUNX1 to drive neurofibromagenesis.** Loss of NF1 elevates RAS-GTP to active downstream MEK/ERK signaling, which in turn phosphorylates RUNX1. Activated RUNX1 drives neurofibromagenesis by inhibiting PMP22, p53/p21 or other unknown gene(s)/pathway(s).



**Fig. 2. NF1 and RUNX1 are both drivers of several solid tumor formation.** NF1 serves as a tumor suppressor to contribute to breast cancer, lung cancer, glioblastoma multiform and several other tumor formation. RUNX1 functions as a tumor suppressor or an oncogene to drive tumorigenesis in the context of specific tissues. NF1 and RUNX1 co-mutations might contribute to tumorigenesis on rare events in some cancers.

## RUNX1 IN NF1-RELATED BLOOD DISEASE

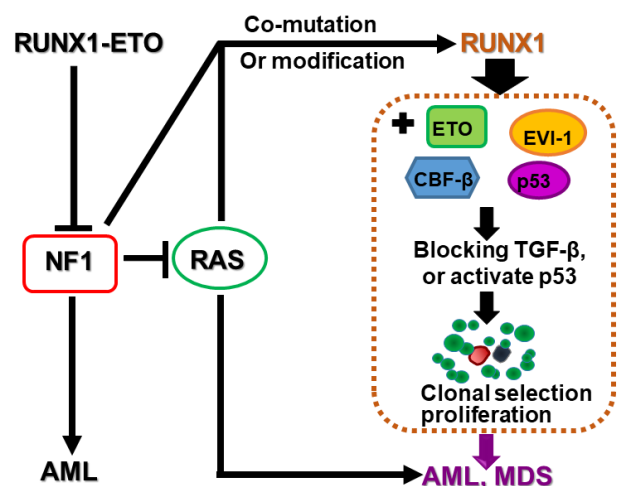
### Acute myeloid leukemia (AML)

Patients with NF1 mutation show a 200- to 500-fold increased risk of JMML, a RAS pathway-driven myeloproliferative neoplasm (Chang et al., 2014). Patients with RUNX1 mutation and/or deletion develop more aggressive AML. RUNX1 somatic point mutations are detected in approximately 15% of adult and 3% of pediatric AML patients (Sood et al., 2017). Microdeletions are detected on chromosomes 17q11.2 and 21q22.12, where the NF1 and RUNX1 genes are located in AML patients (Nakagawa et al., 2011). A report showed that NF1 is transcriptionally repressed by the t(8;21) fusion protein (RUNX1-ETO), suggesting that NF1 is a direct transcriptional target of RUNX1-ETO. Unlike in solid tumors, co-deletion of RUNX1 and NF1 has been proposed to contribute to the molecular pathogenesis of AML (Yang et al., 2005) (Fig. 3).

Several studies suggest that RAS and RUNX1 act in the same pathway to drive the development of AML. Loss of NF1 elevates RAS-MAPK signaling. RUNX1 mutations have a significant association with -7/7q- alteration, and frequently involve receptor tyrosine kinase (RTK)-RAS signaling pathway activation (Niimi et al., 2006). The elevated ERK signaling phosphorylates RUNX1 S276/S293 or affects arginine methylation of R206 and R210 to contribute to the development of AML (Imai et al., 2004) (Fig. 3).

### Myelodysplastic syndromes (MDS)

The initiation and evolution of MDS is driven by genomic events that disrupt multiple hematopoiesis related genes. The frequency of RUNX1 mutations in MDS patients is about 10% (Bejar et al., 2011). Although RUNX1 mutations are suspected to play a pivotal role in the development of MDS, acquisition of additional genetic alterations is also necessary.



**Fig. 3. Co-mutations of RUNX1 and NF1 contribute to blood malignancy.** RUNX1 t(8;21) translocation (RUNX1-ETO) represses NF1 to develop AML. RUNX1 mutation or NF1/RAS-RUNX1 co-mutation develop AML or MDS through clonal selection and cell proliferation.

Bejar et al. (2011) showed that patients with RUNX1 mutations have more mutations of FLT3, N-RAS, PTPN11, or NF1 genes, resulting in a significantly higher mutation frequency for RTK-RAS signaling pathways in RUNX1-mutated MDS/AML patients compared to RUNX1 wild-type MDS/AML patients. A small subset of MDS arise due to deregulation of the RAS pathway, mainly due to NRAS/KRAS/NF1 mutations. In addition, MDS with RUNX1 point mutations is significantly related to hyper-activated RAS signaling pathway (Niimi et al., 2006) (Fig. 3). In a very rare case report, RUNX1 and NF1 co-mutation was detected in a non-langerhans cell histiocytosis patient by whole exome sequencing (Al Mugairi et al., 2019).

## TARGETING RUNX1 OR NF1 FOR THERAPY

Targeting transcription factors, which have traditionally been considered untargetable, is becoming a realistic option with increased understanding of transcription factor biology and technological advances. The interaction between RUNX1 and CBF-B is critical for tumor formation. Therefore, targeting the RUNX1 and CBF-B interaction might be a novel therapeutic strategy for drug development. Increased expression of other RUNX family member may compensate for the antitumor effect elicited by a single RUNX gene silencing suggests that simultaneous attenuation of all RUNX family members might lead to much stronger antitumor effect than suppression of individual RUNX members. Morita et al. (2017) show that targeting RUNX clustering using pyrrole-imidazole polyamides bind to RUNX-binding consensus sites (5'-TGTGGT-3' and 5'-TGCGGT-3') is effective against AML and several poor prognosis solid tumors in mice without notable adverse events. The RUNX1-CBF-B interaction inhibitor, Ro5-3335, preferentially killed human CBF leukemia cell lines, rescued pre-leukemic phenotype in a RUNX1-ETO transgenic zebrafish, and reduced leukemia burden in a mouse CBF-MYH11 leukemia model (Cunningham et al., 2012). Ro5-3335 decreases neurofibroma growth by inhibiting Schwann cell proliferation and inducing cell apoptosis (Hall et al., 2019). During Ro5-3335 treatment, RUNX/CBFB binding sites are blocked and none of the RUNX family member can bind to CBFB to achieve their transcriptional activities.

Plexiform neurofibromas are benign Schwann cell tumors. There is no effective therapy and surgery remains the mainstay of therapy. The MEK inhibitor, Selumetinib, has shown promising efficacy in unoperable plexiform neurofibromas in patients and plexiform neurofibromas in mice, but tumors regrow when drug treatment stops (Dombi et al., 2016; Jensen et al., 2013; Jousma et al., 2015). In mice with Nf1-driven JMML-like myeloproliferative neoplasm, MEK inhibition decreases cell proliferation but does not eradicate disease (Chang et al., 2013). New therapeutic strategies and targets independent of the MAPK pathway are needed for neurofibroma treatment. It will be interesting to test if modulation of the RUNX cluster using the Pyrrole-Imidazole (PI) polyamide gene-switch technology, or other methods, exerts antitumor effects on plexiform neurofibromas. In any case, combinatorial therapies might provide better effects.

## CONCLUSION AND FUTURE PERSPECTIVES

NF1 or RUNX1 mutations are detected in many tumors. NF1 serves as a tumor suppressor while RUNX1 serves as a tumor suppressor or an oncogene in the context of various tissues. NF1 and RUNX1 co-mutations are detected in only rare cancers. Whether relevant NF1 and RUNX1 mutations are mutually exclusive needs to be further studied. The expanded application of next-generation sequencing to cancer patients is expected to identify more RUNX1 or NF1 germline/somatic mutations and other mutations that are known to be somatically mutated in the same or other cancer types. Targeting the MAPK pathway has shown efficacy in NF1 neoplasms but the effects are limited because the MEK inhibitor cause cytostasis not cell death. MEK-independent therapeutic strategies are needed, and testing combinatorial therapies may be useful.

### Disclosure

The authors have no potential conflicts of interest to disclose.

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