

Neurofibromatosis Type 2 Protein, NF2: An Unconventional Cell Cycle Regulator

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Abstract. *Neurofibromatosis type 2 protein (NF2) is an underappreciated tumor suppressor involved in a broad range of nervous system tumors. Inactivation of the NF2 gene leads to neurofibromatosis type-2, which is characterized by multiple benign nervous system tumors and mutations in the gene have been demonstrated in many other tumor types as well. All tumors, regardless of location or grade, lack a fundamental control over cell cycle progression. Historically, NF2 is an unconventional tumor suppressor protein in that it does not directly influence the cell cycle. NF2 links receptors at the plasma membrane to their cytoplasmic kinases to facilitate contact inhibition. However, NF2 can also interact with an array of cytoplasmic and nuclear proteins that affect cell cycle progression. Furthermore, through some of these pathways, NF2 may reverse the functional inhibition of conventional tumor suppressor pathways. Here we review mechanisms utilized by NF2 to regain control of the cell cycle.*

Disruption or inactivation of the neurofibromatosis type-2 (NF2) gene, located on human chromosome 22, leads to the autosomal dominant cancer disorder, neurofibromatosis type-2 (1, 2). Patients with NF2 have a predisposition toward the development of a multitude of CNS tumors, most notably bilateral schwannomas, meningiomas, and ependymomas. Though these tumors are typically benign, they often convey a loss of hearing and balance, paralysis, and can lead to early death (3). While the incidence of NF2 disease is relatively rare, the NF2 protein, also referred to as merlin or schwannomin, has been shown to play a pivotal role in a number of other cancers. For instance, loss or inactivation of the NF2 gene has also been described in highly-aggressive mesothelioma tumors, spontaneous meningiomas and

schwannomas, as well as gliomas and breast cancers (4-9).

The NF2 gene product is a member of the ERM (ezrin, radixin, moesin) family of cell adhesion proteins (10) and as such, NF2 binds either directly or indirectly to the actin cytoskeleton and is essential for the formation of stable adherens junctions (11-14). As a cytoplasmic scaffolding protein, NF2 provides a crucial link between the extracellular environment and cell signaling pathways. Specifically, NF2 prevents the effects of aberrant mitogenic signaling on oncogenic transformation, making NF2 the only member of the ERM family to exhibit tumor suppressor activity (15). At the plasma membrane, NF2 binds to receptor tyrosine kinases and integrins, such as CD44 and $\beta 1$ integrin, respectively (15, 16) which confers responsiveness to cell confluence and growth factor availability to help maintain contact inhibition (15, 17, 18). Consequently, loss of NF2 leads to a loss of contact inhibition and a gain of anchorage independence, further facilitating invasion and mobility of malignantly-transformed cells (19, 20). For a detailed review of these aspects of NF2 function, please see references (19, 20, 37).

Typically, NF2 is concentrated in actin-rich areas of the cell such as filipodia, lamellipodia, membrane ruffles, and cell-to-cell boundaries. Due to its predominant localization to the plasma membrane, it would not appear that NF2 significantly impacts the cell cycle, in contrast to most canonical tumor suppressors. However, several studies have demonstrated the presence of a nuclear fraction of NF2, which would facilitate this protein's ability to impact the cell cycle (21-27), though the presence of nuclear NF2 could merely be indicative of its sequestration during times of rapid growth (20). While NF2 has not been shown to elicit a direct effect on the cell cycle, several studies have demonstrated its influence on cell cycle progression. For example, re-introduction of NF2 induces cell cycle arrest in numerous cells types including, rat and human schwannomas, primary endothelial cells, mesotheliomas, and patient-derived meningiomas (12, 27, 28-33). These studies have led to the speculation that NF2 may indeed regulate progression of the cell cycle across a range of cell types. Below, we review this evidence and its implications in cell cycle regulation and tumor progression.

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Nuclear NF2: In the Right Place at the Right Time

Traditionally, NF2 has been primarily thought of as a cytoplasmic scaffold used to transduce signals from the extracellular *milieu* into the intracellular cues necessary for the cell to form and maintain its boundaries. While this is still considered to be a critical task for NF2, its nuclear localization hints at broader tumor suppressive capabilities. To date, NF2 has been detected in the nucleus of mouse and human glial tumors, osteosarcomas, mesotheliomas, and immortalized schwann cells, to name a few (as summarized in Table 1). While the NF2 gene lacks a canonical nuclear localization sequence, which may make the nuclear localization of NF2 difficult to detect (23), it has been established that NF2 utilizes the CRM1/exportin pathway to exit the nucleus, thereby allowing the use of the CRM1-specific inhibitor, leptomycin B to trap NF2 in the nucleus in cell culture models (22, 23).

The nuclear translocation of NF2 appears to be mediated by both its phosphorylation status and the phase of the cell cycle. NF2 is thought to exist in two conformations, known as the open and closed conformations, which are based on the phosphorylation status of serine residue number 518 (Ser518). Phosphorylation of Ser518, by either p21-activated kinases (PAKs), Rac GTPase, or protein kinase A (PKA), allows NF2 to adopt an open conformation which is considered to be inactive, as the open conformation is unable to inhibit growth *in vitro* (34-36). However, tumor suppressive functions have ascribed to the phosphorylated form of this protein (27). With respect to its nuclear localization pattern, NF2 is retained in the cytoplasm when phosphorylated at Ser518, while the unphosphorylated protein can better-translocate into the nucleus (26, 37-39). Phosphorylated, cytoplasmic NF2 loses its ability to control the cell cycle in schwannoma and mesothelioma cells, suggesting that nuclear translocation of NF2 is critical for its affect on the cell cycle (37, 38).

The subcellular distribution of NF2 is also affected by the phase of the cell cycle (21, 26). The localization of NF2 during the cell cycle has been analyzed in cells synchronized using chemical agents and withdrawal of growth factors, *i.e.* nocodazole and serum starvation, respectively. In the late G₁ phase, NF2 exits the nucleus and is predominantly localized to the cortical membrane during the S and G₂ phases (26). NF2 is then detected in the peri-nuclear region during the G₂/M phase transition, while during mitosis, NF2 associates with the mitotic spindle and remains in the nucleus through early-G₁ phase. However, NF2's nuclear localization may also be dependent on the activity of its binding partners, including PAK and Rac, both of which phosphorylate NF2, and are themselves regulated by the cell cycle.

Since NF2 is typically detected outside of the nucleus, there is a much clearer understanding of its roles and binding partners in the cytoplasm. As a scaffolding protein, NF2 has been shown to bind not only transmembrane proteins at the

cell surface, but also several critical downstream effectors, each of which has distinct influences on the cell cycle.

Cell Surface Signaling Molecules Linking NF2 to the Cell Cycle

B1 integrin and CD44. NF2 has been linked to many receptor types at the plasma membrane to propagate their extracellular signals. For example, integrins have a strong influence on cell cycle progression, primarily through the induction of cyclin D1 and repression of p21 and p27 (40). NF2 binds to β 1 integrin in Schwann cells (Figure 1), perhaps allowing for the signal transduction cascade triggering differentiation and/or preventing oncogenic transformation (16). All ERM family proteins, especially NF2, bind the cell surface glycoprotein, CD44 (10, 15, 41) which is linked to the cell cycle in that it enhances cyclin E expression, and in turn suppresses that of p21 and p27 (42). When bound to NF2, for example, CD44 is unable to transduce growth-promoting signals in response to its ligand, hyaluronate (15).

ErbB receptors. NF2 has profound effects on several members of the ErbB family of receptor tyrosine kinases (RTKs) including EGFR, ErbB2, and ErbB3. In fact, NF2 utilizes a multifaceted approach to disrupt signaling through these cell surface molecules. For example, NF2 binds to EGFR (Figure 1), *via* NHE-RF1, and retains it in an inactive state, thereby blocking signaling to its downstream effectors, including Src, Raf, ERK, or Akt. Alternatively, NF2 can inhibit the internalization and recycling of activated EGFR molecules as well as trafficking of new EGFR subunits to the cell surface (14, 43-45). Inhibition of EGFR, a potent modulator of cell cycle progression through its inhibitory effects on p27, in addition to its enhancement of cyclins A, D2, and E (46), promotes G₁ arrest; however it has yet to be shown whether NF2 can also promote G₁ arrest *via* an EGFR-dependent method (46, 47). Inactivation of the ErbB members, ErbB2 and ErbB3, by NF2 precludes the binding of ErbB2 to its downstream effector, Src, and prevents further activation of FAK and paxillin (48), while also preventing activation of protein kinase B (PKB/Akt) and the mitogen-activated protein kinases, ERK1 and ERK2 in Schwann cells (14). FAK and paxillin both suppress the essential cell cycle inhibitors, p21 and p27 (49, 50). Additionally, paxillin enhances the expression of the transcription factor c-Jun, an oncoprotein which induces cyclins A and D1 (51, 52). Consequently, NF2-deficient schwannoma cells display an over-abundance of EGFR, ErbB2, and ErbB3 on the cell surface and up-regulation of their downstream effectors, which also serves to drive cellular proliferation (14-16).

PDGFR. Platelet derived growth factor receptor (PDGFR) molecules, another group of RTKs, also accumulate on the

Table I. Evidence for nuclear localization of NF2.

Regulation of nuclear translocation	Reference	Detection method*	Cell types examined	Species
Phosphorylation of Ser518	Lü <i>et al.</i> (2008) (38)	IF and IHC	Neurons	Human
	Thaxton <i>et al.</i> (2007) (39)	IF	Schwannoma	Human
	Li <i>et al.</i> (2010) (27)	IF	Mesothelioma	Human
			Squamous carcinoma	
			Schwann (immortalized)	
G ₁ phase of cell cycle	Muranen <i>et al.</i> (2005) (26)	NT, IF, NF, WB IF, NF, WB NF, WB	Mammary epithelial	
			Umbilical vein endothelial	
			Schwannoma	Mouse
			Glioma	Human
			Osteosarcoma	
Unknown	Kressel and Schmucker (2002) (23) Shollar <i>et al.</i> (2004) (25) Beltrami (unpublished)	NT and IF IF, IHC, NF, WB IHC	Embryonal kidney	
			Embryonic fibroblasts	Mouse
			Malignant peripheral nerve sheath	Mouse
			Glioblastoma	Human

*IF: Immunofluorescence; IHC: immunohistochemistry; NF: nuclear fractionation; NT: nuclear trapping; WB: western blotting

surface of NF2-deficient schwannoma cells (14, 53). Activation of these receptors facilitates the phosphorylation of β -catenin by either Src or the Rac/PAK2/JNK cascade (Figure 1). Upon phosphorylation of β -catenin by Src, β -catenin dissociates from the complex and de-stabilizes the adherens junctions. Further, phosphorylation of β -catenin by the Rac/PAK2/JNK pathway allows for the translocation of β -catenin into the nucleus, where it acts as a positive transcription factor for c-myc and cyclin D1, thus enhancing the cell cycle (54). In order to form stable adherens junctions which maintain cell-cell boundaries, NF2 complexes with β -catenin and either E or N cadherin (54, 55). The localization of NF2 to the plasma membrane appears to be crucial in the maintenance of contact inhibition. In its role as a scaffold, NF2 thus prevents the oncogenic effects of aberrant signaling between receptors and their kinases. Independent of receptor activation, these cytoplasmic kinases have significant ties to cell cycle, providing another level to which NF2 can antagonize cell cycle progression.

Cytoplasmic Kinases as Intermediates Linking NF2 to the Cell Cycle

Rac1 and *Cdc42*. Earlier studies have highlighted the importance of the Rho GTPases, Rac1 and Cdc42, in facilitating cell cycle progression. Specifically, these kinases are capable of enhancing both cyclin D1 expression and the transcriptional activity of E2F proteins. All of these events ultimately promote the G₁ to S phase transition of the cell cycle (56, 57). While NF2 does not directly bind to Rac1, it serves to prevent Rac1 activation, possibly by inhibiting key GDP-GTP exchange *via* Rac1-associated guanine exchange factors

(GEFs) (58, 59) (Figure 2). NF2 can also function upstream of Rac1, *via* the tight junction protein, Angiomotin, and GTPase activating protein, Rich1, to prevent Rac1 activation (60). Rac1 activation is required for its localization to the plasma membrane where it allows cells to escape contact inhibition. Expression of NF2 prevents this phenomenon, and has been also shown to obstruct the motility of Rac1-transformed cells (18, 31). Interestingly, a reciprocal interaction has also been observed, whereby Rac1 can phosphorylate NF2 at Ser518, thus forcing NF2 into an open conformation which detaches it from the cytoskeleton (18). Rac1 is also activated in response to integrin engagement to promote the expression of cyclin D1 and progression of endothelial cells to the G₁ phase of the cell cycle (61). Consequently, enhanced NF2 expression can negate G₁ phase progression in schwannomas, which have been previously shown to express aberrant Rac1 activation (59, 62, 63). Cdc42 is also capable of inactivating NF2, but it is unclear whether this is a direct consequence of Cdc42 or requires Rac1 to mediate this effect (18). Unlike its ability to inhibit Rac1, NF2 is unable to block the activation of Cdc42. Nevertheless, NF2 can prevent Cdc42 interaction with its downstream effectors, thus blocking mitogen-activated protein kinase (MAPK) signaling (62-65). Thus, redundancy and overlapping among NF2 and Rac1/Cdc42 can either promote growth or prevent proliferative signaling, depending on their phosphorylation status (20).

PAKs. PAKs are commonly considered downstream effectors of Rho GTPases, since phosphorylation by both Rac1 and Cdc42 are required for their activation (24, 66, 67). Several studies have shown that PAKs 1 and 2 are required in order for Cdc42 and Rac1 to phosphorylate NF2 (24, 34) (Figure 2).

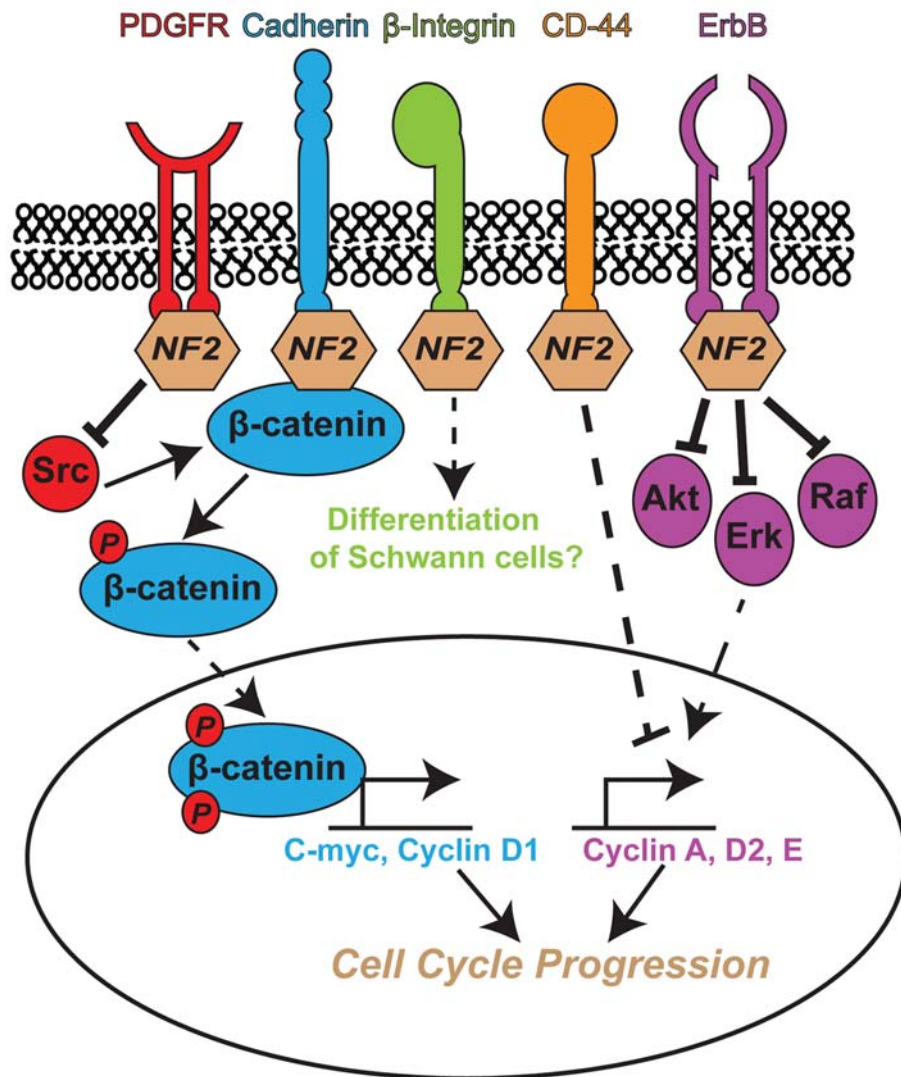


Figure 1. Interaction of NF2 with cell surface receptors can alter cell cycle progression through several signaling pathways. NF2 is predominantly localized to the plasma membrane where it interacts with various cell surface receptors, each with an established effect on the cell cycle. Through its interactions with PDGFR and Src or cadherins, NF2 prevents the phosphorylation of β -catenin. In doing so, β -catenin cannot translocate to the nucleus and act as a positive transcription factor for cell cycle enhancers, c-myc and cyclin D1. In a less understood interaction, NF2 binds to β -integrins to promote the differentiation of Schwann cells, which may result in the halting of the cell cycle. Similar to all ERM proteins, NF2 interacts with CD-44, perhaps to act as a negative regulator of cyclins A, D2, and E. NF2 also networks with ErbB receptors 1, 2, and 3, and acts as an inhibitor of Akt, ERK, and Raf. This interruption of ErbB signaling may disrupt the effects of these receptors on cell cycle progression.

However, PAKs can also act upstream of Rac1 to facilitate inactivation of NF2 (31). As seen with Rac1, PAKs also maintain a reciprocal relationship with NF2. By binding to PAK1, NF2 can hinder Rac1/PAK1 interaction and subsequent activation of PAK1 (68). PAKs 2 and 3 are also subjected to inactivation by NF2, most likely *via* the same mechanism (13, 24, 69, 70). In turn, PAKs can also contribute to the functional inactivation of NF2, and successive loss of contact inhibition (18, 24, 34, 66). Regardless of where PAKs lie within this

cascade, a significant relationship clearly exists between PAKs and NF2. Some studies suggest that NF2-deficient tumors display an “oncogene addiction” to PAKs. For instance, in rat schwannoma cells, which require activated PAK1 for survival, cells infected with viral vectors inducing shRNA-mediated suppression of PAK1 were capable of methylating the promoter of this foreign construct, thus preventing PAK1 extinction (69). Elevated expression of group 1 PAKs, including PAKs 1-3, has been seen in primary schwannomas from NF2 patients and use

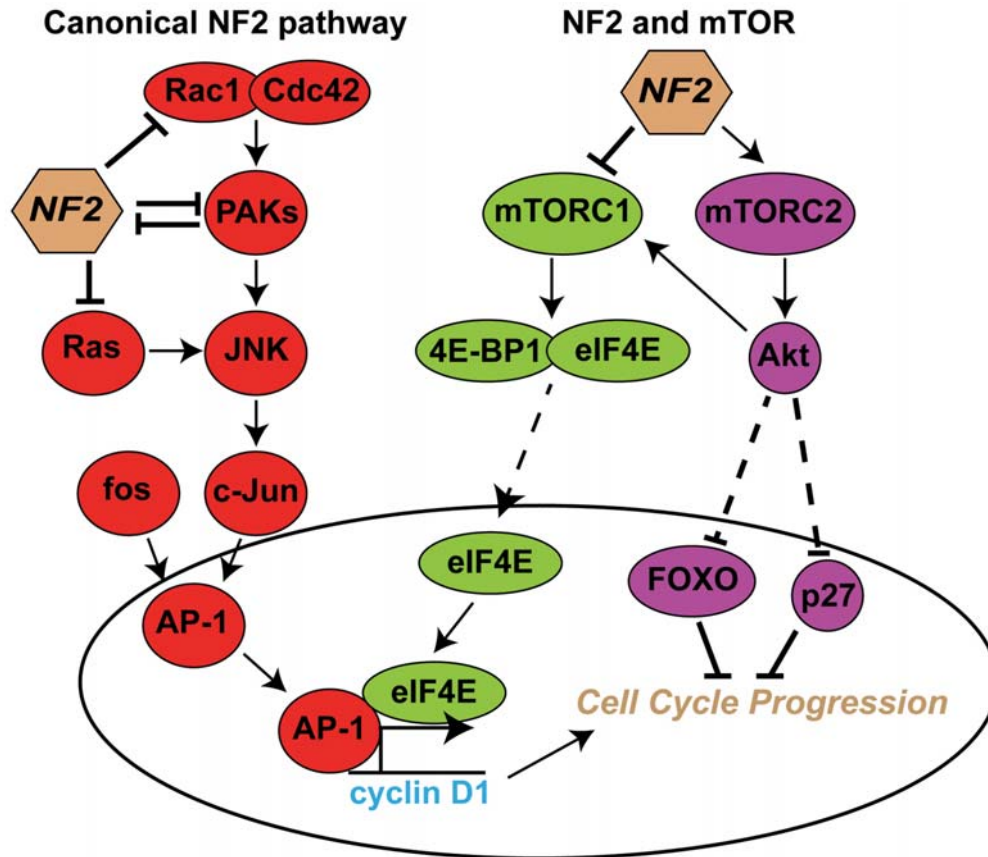


Figure 2. The canonical NF2 pathway and an alternative pathway via mTOR for affecting the cell cycle. In the cytoplasm, NF2 is known to disrupt the downstream signaling of Rac1 and Cdc42 at several points. In addition to directly inhibiting the Rac1/Cdc42 complex, NF2 can also inhibit activation of PAKs, and Ras. In either exchange, NF2 prevents the activation of JNK and c-Jun, and the assembly of the AP-1 transcription complex. A reciprocal interaction also occurs in this pathway whereby PAKs can inhibit the actions of NF2. Interestingly, NF2 has differential effects on mTORC1 and mTORC2. By activating mTORC2, NF2 allows activation of Akt and its subsequent repression of FOXO and p27, known inhibitors of cell cycle progression. However, NF2 inhibits the activation of mTORC1 and subsequent dissociation of transcription factors, 4E-BP1 and eIF4E. When freed, eIF4E, along with AP-1, can amplify the transcriptional activation of cyclin D1, demonstrating the overlapping nature of these separate pathways.

of a group 1-specific inhibitor peptide significantly hindered several oncogenic properties of cells lacking functional NF2 (70, 71). NF2-deficient tumors may also depend on activated PAKs for survival because of their multifaceted effects on cell cycle progression. For example, PAK1 is the intermediate linking Ras to enhanced cyclin D1 expression (72), which promotes progression from the G₁ to S phase of the cell cycle. In addition, late in S phase, PAK1 phosphorylates histone H3 to allow for chromatin condensation of newly-synthesized DNA73 and also serves to activate Aurora A and polo-like kinase 1 (plk1), which are crucial for the G₂/M transition of the cell cycle (74, 75). Furthermore, PAK1 translocates to the nucleus during mitosis, where it plays an active role in assuring the proper segregation of chromosomes (73). Taken together, these studies demonstrate several key points at which NF2-mediated inhibition of PAKs, in particular PAK1, could halt cell cycle progression.

JNK. The MAPK, c-Jun N-terminal kinase (JNK) is a seemingly under-represented, downstream effector of the Rac1/PAK cascade. JNK is activated by Rac1 directly or *via* PAK1; however this process can be ameliorated in the presence of NF2 (18, 56). Earlier studies detected enhanced JNK 1 and 2 activation in NF2-deficient tumors, which was assumed to be simply an indication of Rac and PAK activity (18, 30, 62) (Figure 2). However, one could argue that JNK itself provides an important link between NF2 and the cell cycle. For example, the transcription factor c-Jun, a primary target of JNK activation, is freed to homo- or heterodimerize with a fos protein (*i.e.* c-fos, FosB, Fra1, or Fra2) upon phosphorylation by any of the JNK isoforms to form the activator protein 1 transcriptional complex (AP-1). In NF2-deficient cells, JNK overactivation correlates with enhanced transcriptional activity by c-Jun and AP-1 (18, 30). Therefore, it is not surprising that JNK-mediated signaling *via* c-Jun and

AP-1 results in enhanced cyclin D1 expression in NF2-null cells, as the cyclin D1 promoter is up-regulated by both of these proteins (30, 76). While this link between JNK activation and cell cycle progression is well-established, it is not yet known whether silencing of JNK in NF2-deficient cells is capable of triggering cell cycle arrest.

Ras. Of the oncogenic kinases described here, NF2 and Ras, a member of the small GTPase family, have the longest-standing history. In fact, the ability of NF2 to inhibit Ras-mediated oncogenic transformation of NIH3T3 cells was the first report of NF2's tumor suppressor activity (77) (Figure 2). While NF2 has not been shown to directly bind Ras, it significantly hinders its downstream signaling (59) by preventing Ras up-regulation of AP-1 activity and cyclin D1 expression (78). NF2 also prevents Ras-mediated activation of JNK, which results in suppression of AP-1 and cyclin D1 expression (70). In response to serum stimulation, Ras phosphorylates ERK, which promotes its translocation to the nucleus, where nuclear ERK can then act as a transcriptional activator of genes which contain serum-responsive elements (SREs) within their promoters. One of these genes, *Elk1*, can in turn promote the expression of cyclin D1 as well as G₁ phase progression. NF2 is able to ameliorate these effects by preventing Ras-mediated ERK phosphorylation (59, 79). Despite the well-characterized effects of NF2 on Ras-mediated oncogenicity, it has yet to be determined whether NF2 overexpression can, in fact, enable Ras-transformed cells to regain control of the cell cycle.

mTOR. Aberrant signaling mediated by the serine/threonine kinase mammalian target of rapamycin (mTOR) is a characteristic of numerous malignancies, including those arising from the inherited cancer disorders, neurofibromatosis type 1 and tuberous sclerosis complex (80). mTOR is a member of two distinct complexes, mTOR complex 1 and 2 (mTORC1 and mTORC2, respectively). As a member of both complexes, activation of mTOR is influenced by growth factor availability, making it a key regulator of proliferation in the tumor microenvironment (81). NF2 has differing effects on mTORC1 and mTORC2, further highlighting the complexities of mTOR-mediated signaling (Figure 2). Upon stimulation by either growth factors or nutrients, mTORC1 assembles and promotes the phosphorylation of its two key effectors, p70 S6 kinase (S6K1) and eukaryotic initiation factor 4E (eIF4E) binding protein 1 (4E-BP1). Activation of S6K1 is not required, but certainly aides in cell cycle progression in response to mTORC1 signaling (82). However, the mechanism and necessity of S6K1 involvement in cell cycle progression is still under debate (83). In parallel with S6K1 activation, phosphorylation of 4E-BP1 results in the release eIF4E protein, which consequently activates genes responsible for promoting the G₁ to S phase progression, mainly *via* cyclin D1 (84). NF2 has been shown to be a potent inhibitor of mTORC1 signaling in mesotheliomas,

meningiomas, and schwannomas as mTORC1 activation is triggered by integrin-mediated loss of NF2 or in its absence. Consequently, many NF2-deficient cell lines display constitutive mTORC1 activation, as assessed by excessive phosphorylation of S6K1 and 4E-BP1 and expression of cyclin D1. Interestingly, only NF2-deficient cells are sensitive to growth inhibition by the classic mTORC1 inhibitor, rapamycin (33, 85, 86). These studies provide compelling evidence for the use of NF2 status to determine the responsiveness of a tumor to mTOR inhibitors.

Unlike mTORC1, mTORC2 is only responsive to growth factor stimulation and is not inhibited by treatment with rapamycin. In response to IGF1, EGF, or PDGF stimulation, NF2 activates mTORC2 in Schwann and arachnoid cells, the cells of origin for schwannomas and meningiomas, respectively (86). However, the mechanism behind mTORC2 activation has not yet been elucidated. To propagate mTORC2 signaling, Akt1 (also referred to as protein kinase B) is phosphorylated by this complex and, accordingly, Akt1 activation is impaired in cells in which NF2 is silenced (85). Interestingly, Akt1 and S6K1 are both members of the AGC kinase family, demonstrating a certain homology shared by mTORC1 and mTORC2 substrates (84). The consequences of Akt1 activation of NF2-deficient cells have not yet been determined, however, Akt1 activation is typically associated with cell cycle progression. For instance, active Akt1 negatively regulates the cdk inhibitor, p27, as well as the transcription factor, FOXO. Akt1 also targets the E3 ubiquitin ligase, HDM2 (the human homolog of MDM2), to promote the degradation of p53 (84). While this link between NF2 and Akt1 activation may seem counterintuitive to the role of NF2 as a cell cycle inhibitor, some speculate that activation of the mTORC2 may compensate for the inactivation of mTORC1 in these malignancies (86). For instance, activation of Akt1 *via* mTORC2 can lift repression of mTORC1 activation (81). Similar to NF2, the tumor suppressor proteins of the tuberous sclerosis complex (TSC) cancer disorder, TSC1-TSC2, inhibit mTORC1 and activate mTORC2 signaling (87). This pattern of differential regulation of the mTOR complexes may indicate a novel approach toward cell cycle regulation through mTOR signaling.

In response to a multitude of extracellular clues, the delicate balance is tested between NF2 and several of its cytoplasmic binding partners. As highlighted above, NF2 combats the activation of Rac1, Cdc42, PAKs, JNK, and Ras to promote growth arrest (Figure 2). In turn, NF2 is inhibited by Rac1, Cdc42, and PAKs to restore cellular proliferation. NF2 has also an interesting relationship with mTORC 1 and 2. Similar to another tumor suppressor protein, TSC, NF2 activates mTORC2 while suppressing mTORC1, which appears counterintuitive to its potential role as a cell cycle inhibitor. Collectively, these cytoplasmic kinases drive cell cycle progression, providing key targets through which NF2 can restore cell cycle integrity. Alternatively, these cytoplasmic interactions could offer a second line of defense in cells

containing either mutant receptors or NF2, which are therefore unable to form stable cellular junctions. As described earlier, NF2 can also shuttle from the cytoplasm to the nucleus, depending upon its phosphorylation status and stage of the cell cycle. Its role in the nucleus is only currently being recognized as part of its tumor suppressive function through its binding and down-regulation of a few potent oncoproteins.

NF2 Interacts with Nuclear Oncoproteins

CRL^{DCAF1}. Though the post-translational modification of ubiquitination occurs in the cytoplasm, ubiquitin ligases have more recently gained appreciation for their effects on the cell cycle. One such ubiquitin ligase is CRL4^{DCAF1}, a member of the cullin-ring family of E3 ligases. In this multi-subunit complex, the adaptor (DDB1) and scaffolding (Cullin-4) subunits interact with the substrate acceptor (DCAF1). DCAF1 was first identified as the cellular binding protein for the HIV/SIV protein, Vpr, and thus originally named Vpr binding protein (VprBP). Even though Vpr is not required for HIV/SIV replication it is a heavily-conserved protein among various strains and can enhance viral pathogenesis. Vpr binds to DCAF1, at the G₂ phase of the cell cycle, to cause cycle arrest, which would make cells more permissive to viral infection (88). However, in the absence of Vpr proteins, DCAF1 is still capable of appreciably altering cell cycle progression (89). Activation of the CRL4^{DCAF1} complex also permits cell cycle progression by regulating expression of ubiquitin ligases that target histones, or by recruiting chromatin remodeling enzymes (89, 90). Recently, DCAF1 was identified as a nuclear binding partner for NF2. Whether this triggers the proteasomal-mediated degradation of NF2 is still under debate (27, 91). The interaction between DCAF1 and NF2 occurs only when NF2 is in its closed conformation and prevents the ubiquitin ligase function of the CRL4^{DCAF1} complex. As a consequence, NF2 prohibits schwannoma cells from progressing through G₁ and into S phase. Interestingly, this same effect was not seen in Schwann cells indicating that DCAF1 is essential for rapid cell cycling in transformed, but not normal cells (27). While this study has provided a greater appreciation for NF2's role in the nucleus, it is still unclear which cell cycle regulators NF2 specifically alters through the inhibition of the CRL4^{DCAF1} complex.

JCV T-antigen. Interest in the relationship between NF2 and the JC virus (JCV) oncogenic protein T-antigen, resulted from previous studies showing an association between these two proteins in a mouse model of peripheral nerve sheath tumors (25). JCV is a human polyomavirus, and the causative agent of the rare, but fatal demyelinating disease, progressive multifocal leukoencephalopathy, PML (92). In addition to its role in PML pathogenesis, JCV has exhibited oncogenic potential in cell culture and experimental animal models and has been detected in human medulloblastomas,

astrocytomas, ependymomas, meningiomas, and numerous other mixed-lineage gliomas (93). Similarly, the simian homolog to JCV, SV40, has also been linked to human malignancies such as mesothelioma, meningiomas and various grade of gliomas (94, 95). Due to the great variability of the detection of these viruses in human tumors, the causal role of JCV and SV40 in human malignancies is still heavily debated (93). Taken together, the seemingly reciprocal relationship of NF2 and JCV in these overlapping tumor types has led our group to investigate whether NF2 can combat the oncogenic effects of polyoma viruses. To promote JC viral replication, T-antigen sequesters key cell cycle moderators, including p53 and Rb. JCV T-antigen binds to wild-type p53, exclusively (96) and, in fact, was first identified as a consequence of its binding to SV40 T-antigen (97). Furthermore, the ability of JCV T-antigen to bind Rb is necessary for its transformative behavior (98). JCV T-antigen also enhances oncogenic signaling through the Wnt pathway, by stabilizing key members, such as β -catenin and c-myc (99, 100) where T-antigen expressing cells contain higher levels of transcriptionally active β -catenin, as well as c-myc and cyclin D1 (101).

JCV T-antigen transgenic mice develop a broad range of neuronal and glial-origin tumors, in particular, malignant peripheral nerve sheath tumors, similar to those seen in neurofibromatosis type-1 patients. Within these tumors an interaction between NF2 and T-antigen was detected in the nucleus of a subset of tumor cells (25) and is capable of promoting the down-regulation of T-antigen in human glial and neuronal cells (Beltrami, unpublished observation). While the down-regulation of T-antigen expression by NF2 was able to ameliorate JCV promoter activation, the potential impact of NF2 on p53 and Rb, or Wnt signaling pathways has not yet been established. More recently NF2 is gaining appreciation for its role in the nucleus of tumor cells. While there is an incomplete understanding of its function there, a review of nuclear NF2 literature has revealed an underlying theme of interactions between NF2 and proteins known to promote viral pathogenesis. Similar to most canonical oncogenes, viral proteins and their cellular targets (T-antigen, and CRL^{DCAF1}, respectively), have evolved to force cell cycle progression of infected cells, in order to obtain the necessary machinery for further viral replication. Through the antagonism of such viral associated proteins, NF2 can play a pivotal role allowing both viral infected and transformed, uninfected cells to regain control of the cell cycle.

Convergence of NF2 with Classical Tumor Suppressor Pathways

p53. The most commonly mutated or lost tumor suppressor protein in cancer, p53, has extensive ties to the cell cycle (102). In brief, p53 is a potent inducer of G₁ phase arrest,

via its transcriptional activation of anti-proliferative genes, including p21, Noxa, Puma, and Perp (103). While a direct interaction among NF2 and p53 has not been reported, previous literature has suggested that potential tumor suppressive synergy may exist between these two proteins. In fact, the NF2 and p53 alleles map to the same locus in the mouse genome, and loss of both genes leads to the development of highly metastatic tumors including osteosarcomas, malignant peripheral nerve sheath tumors, choroid plexus carcinomas, and mesotheliomas (104, 105). In addition, human meningiomas displaying NF2 loss of heterozygosity (LOH) and p53 mutations are typically of a higher grade than those with only NF2 LOH (106). Furthermore, p53 expression can overcome the oncogenic consequences of loss of NF2 in neural crest tumors, indicating that p53-specific therapies could be beneficial in NF2-deficient tumors (107). These results are not surprising since NF2 has been shown to contribute to the stabilization of p53 through the down-regulation of Mdm2 which also results in the enhancement of p21 promoter activity and sensitization of cells to p53-induced apoptosis (108). The possibility remains, however, that NF2 also interacts with the p53 downstream effector, p21, which provides a potential link between the influence of both NF2 and p53 on cell cycle progression.

Rb. Retinoblastoma (Rb), the first described tumor suppressor protein, is a central negative regulator of cell cycle progression (109). Rb is considered to function as a “pocket-protein”, whereby it binds to members of the E2F family of transcription factors, thus preventing the E2Fs from acting on downstream target genes which promote the cell cycle from G₁ to S phases, including cyclins E and A, thymidine kinase, and DNA polymerase- α . When Rb is activated *via* phosphorylation by cyclin-dependent kinases, E2F is released, freed to heterodimerize with a binding partner (DP), leading to transcriptional activation. In addition to its role in sequestering E2F, Rb can also recruit histone deacetylase-1 (HDAC1) to condense chromatin and hinder transcription of a given target promoter (98). Though an interaction among NF2 and Rb has not been reported, several NF2 binding partners can negatively impact the function of Rb. For example, activation of EGFR, Rac1, or Cdc42 initiates a cascade resulting in the phosphorylation of Rb (46, 56, 57). Cytoplasmic NF2, has been demonstrated to prevent Ras-mediated phosphorylation of Rb and transcriptional activation of E2F78 while nuclear binding partners of NF2, including DCAF1 and T-antigen, can force S-phase entry by phosphorylation or sequestration of Rb, respectively (89, 98). This mechanism of sequestering Rb can also be seen with human papillomavirus E7, and adenovirus E1A proteins, further solidifying a central role for Rb inactivation as an essential step in viral oncogenesis (98).

Conclusion

Traditionally, NF2 is typically viewed as a scaffolding protein primarily located at the plasma membrane, where it prevents excessive signaling *via* several cell surface receptors and their cytoplasmic kinases. However, NF2 inactivation is seen in an array of tumors, all of which share a dysregulation of the cell cycle. Here, we provided several lines of evidence suggesting that NF2 could prevent cell cycle progression set forth by these receptors, and serve as an unconventional tumor suppressor protein with many potential mechanisms by which it may halt the cell cycle. A fraction of NF2 has also been shown to interact with many key signal transduction pathway members in the cytoplasm as well as oncoproteins in the nucleus. The outcome of these multi-layered pathways is the inactivation of conventional tumor suppressors, such as p53 and Rb, consequently leading to cell cycle progression. In considering all of these interactions, it is likely that cell cycle control is the resulting downstream consequence of NF2 function. As such, NF2 based therapeutics may provide a dual approach, whereby its traditional role as suppressor of invasion and metastasis can be targeted, as well as its proposed action as a cell cycle regulator.

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