

Interferon induction held captive in tumour cells

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Unusual nucleic acids activate innate immunity and may be present in transformed cells. Meng et al. (2021) find that cancer-associated mutations in *NF2* turn this tumour-suppressor into a potent antagonist of DNA- and RNA-induced innate immune signalling.

The type I interferon (IFN) system is a first line of cellular defence. It is best known for mediating host protection against viruses but also responds to other disturbances of homeostasis. For example, type I IFNs play important and multifaceted roles in malignant disease. Expression of type I IFNs is triggered following detection of foreign or unusual nucleic acids ([Bartok and Hartmann, 2020](#)). Several distinct receptors sense RNA or DNA in different subcellular compartments. They then signal for type I IFN gene induction via a common pathway involving the kinase TBK1 and the transcription factor IRF3. In this issue of Molecular Cell, Meng et al. (2021) report that mutated forms of the tumour suppressor NF2 subvert type I IFN induction by sequestration of TBK1 and IRF3. This blunts type I IFN induction and may facilitate cancer progression ([Meng et al., 2021](#)).

NF2 (also known as Merlin and schwannomin) is an upstream regulator of the Hippo pathway that controls the growth of tissues ([Harvey et al., 2013](#); [Zheng and Pan, 2019](#)) (Figure 1A). Mutations in *NF2* are found in cancers affecting the nervous system, such as schwannomas and meningiomas, and in tumours originating from other organs ([Petrilli and Fernandez-Valle, 2016](#)). Given previously identified functions of the Hippo pathway in type I IFN induction ([Jiao et al., 2018](#); [Zhang et al., 2017](#)), Meng et al. (2021) now explored whether NF2 plays a role in nucleic acid sensing. The authors selected three known missense mutations found in cancer patients that are located in NF2's FERM domain. Surprisingly, reconstitution of NF2-knockout cells with mutant NF2 (mNF2) profoundly blocks nucleic acid sensing. Normally, following engagement of nucleic acid sensors, TBK1 and IRF3 are phosphorylated, type I IFN and IFN-stimulated genes are induced and cells become refractory to virus infection (Figure 1B and C). All of these effects are strongly diminished in cells expressing mNF2 in response to a variety of stimuli. These include the synthetic RNA poly I:C, cGAMP (the second messenger in the cytosolic DNA sensing pathway), vesicular stomatitis virus infection and overexpression of the adaptor proteins MAVS and STING.

The authors then uncover the molecular and cellular basis of this inhibition. Comparison of wild-type NF2 and mNF2 by immunofluorescence imaging revealed distinct subcellular localisations: NF2 is predominantly located at plasma membrane and associated with the cytoskeleton whereas mNF2 shows a cytoplasmic distribution. Moreover, upon overexpression of MAVS or STING, which mimics activation of the RNA and DNA sensing pathways, respectively, mNF2 but not wild-type NF2 forms punctate structures. Live-cell imaging experiments suggest that these structures are molecular condensates formed by liquid-liquid phase-separation (LLPS). LLPS is defined as formation of droplets that undergo

fusion and fission, exchange materials with the cellular environment and exhibit fluorescence recovery after photobleaching ([Xiao et al., 2021](#)), all exhibited here.

Interestingly, these condensates contain IRF3 and were found not only in cell lines but also in vestibular schwannomas from a subset of patients with *NF2* mutations. Domain mapping experiments revealed that mNF2 binds via its FERM domain to the IRF association domain of IRF3 and via its hydrophilic C-terminal tail (CTT) to the double coiled-coil domain of TBK1. These interactions and formation of mNF2 aggregates require TBK1 kinase activity, IRF3 and an upstream trigger. Notably, the phosphorylation pattern of IRF3 is altered in cells expressing mNF2, which the authors attribute to the presence of phosphatases such as the RACK1-PP2A complex in mNF2 condensates. These data together with structural modelling suggest that NF2 mutations result in conformational changes exposing the FERM domain and CTT, which then interact with activated IRF3 and TBK1, resulting in the formation of a large molecular condensate entrapping IRF3, and ultimately inhibition of type I IFN induction (Figure 1D-F).

It is interesting to note that phase separation is an emerging concept in immune signalling ([Xiao et al., 2021](#)). For example, the DNA sensor cGAS undergoes LLPS together with immunostimulatory DNA ([Du and Chen, 2018](#)). However, rather than playing an inhibitory role, LLPS of cGAS facilitates DNA sensing. It is likely that future work into how phase separation regulates innate immune responses, positively and negatively in different contexts, is going to be fruitful.

Another interesting area for future investigation is the cross-regulation of the Hippo pathway and type I IFN induction ([Wang et al., 2020](#)). Indeed, Meng et al. (2021) show that wild-type NF2, in opposition to mNF2, moderately enhances TBK1/IRF3 signalling. Moreover, it is conceivable that the conformational changes triggered by NF2 mutations might also occur temporarily in the wild-type protein to provide a negative feedback loop. Post-translational modifications or association with accessory proteins could mediate the change in configuration. If this is indeed true, it would be interesting to screen for small molecules targeting NF2's conformation to control type I IFN induction in disease.

Meng et al (2021) further explore the role of mNF2 in anti-tumour immunity using two *in vivo* models. In one, melanoma cells expressing a constitutively active version of STING were transplanted into mice. These cells form only small tumours that are infiltrated by CD4 and CD8 T cells and macrophages. Additional expression of mNF2 in the grafted melanoma cells restores tumour growth and diminishes immune cell infiltration. These observations point to a tumour-cell intrinsic role of mNF2. The second model interrogates tumour-cell extrinsic effects and involves intramuscular cGAMP treatment of mice bearing melanomas formed by wild-type cells. In this setting, cGAMP has an anti-tumour effect, but this response is reduced in transgenic mice expressing mNF2.

The precise roles of mNF2 in cancer development and progression warrant further investigation. Proliferation of transformed mNF2 cells is likely a combined effect of the inhibition of innate immune responses and subsequently reduced immune cell recruitment to the tumour and the absence of wild-type NF2 that inhibits cell proliferation via the Hippo pathway. The relative contributions of these mechanisms may vary between different types of tumours with *NF2* mutations.

Finally, it is tempting to speculate that tumour cells devoid of a functional nucleic acid sensing machinery, such as in the presence of *NF2* mutations, may be an ideal target for oncolytic virotherapy. Indeed, Meng et al. (2021) show that the number of vesicular stomatitis virus-infected cells is significantly increased upon mNF2 reconstitution. However, reduced

immune cell recruitment into mNF2 tumours may indicate a need for genetically engineered oncolytic viruses that elicit improved immune cell infiltration, for example by expressing cytokines.

Figure legend.

Figure 1: Mutant NF2 entraps IRF3 by liquid-liquid phase-separation.

(A) Wild-type NF2 is an upstream regulator of the Hippo pathway that controls the activity of YAP/TAZ by phosphorylation. Active, unphosphorylated YAP/TAZ promotes cell growth and inhibits innate immunity. (B) Upon signalling from upstream nucleic acid sensors, the adaptor proteins STING, MAVS and TRIF trigger phosphorylation and activation of TBK1. (C) Active TBK1 then phosphorylates IRF3, which induces type I IFNs. (D) NF2 mutations found in cancer change NF2's conformation and expose an IRF3 binding interface. (E) IRF3-mNF2 interactions result in the assembly of a complex containing mNF2, IRF3, TBK1 and the phosphatase PP2A. (F) This complex oligomerises and forms a liquid-liquid phase-separated condensate within the cytoplasm that imprisons and inactivates TBK1 and IRF3.

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