CANCER

How to lose tumor suppression

Dominant-negative effects alone explain the spectrum of p53 mutations in myeloid cancer

By **David Philip Lane**[1,2]

The tumor suppressor gene, *TP53*, is the most commonly mutated gene in human cancer. The resulting loss of activity of the encoded protein, p53, causes genetic instability and resistance to chemotherapy. *TP53* mutations are mostly single-nucleotide point mutations that alter one amino acid, rather than deletions. These missense mutations can cause loss of function (LOF) but do they also exert dominant-negative effects (DNEs) and confer additional neomorphic gain of function (GOF) to p53? On page 599 of this issue, Boettcher *et al.* (*1*) present a comprehensive analysis of the effects of 7860 p53 point mutations in myeloid malignancies. They find clear evidence for LOF and DNEs but no evidence for GOF because the DNEs select for the spectrum of mutations found in human myeloid malignancies. Therefore, p53 GOF does not explain the mutational spectrum of *TP53* despite strong evidence for their importance in mice (*2*).

Classically, oncogenes are created by mutations in proto-oncogenes that enhance their biochemical activity. These mutations tend to repeatedly occur at precise amino acid positions—for example, the Glu[12]Asp mutation found in the KRAS oncoprotein locks the protein in the activated form. By contrast, mutations in tumor suppressor genes result in LOF that are typically associated with deletions and mutations that create premature stop codons and thus truncation or loss of the encoded protein (*3*). Although *TP53* is a tumor suppressor gene, mutations are typically missense mutations that affect amino acids throughout the central DNA-binding domain (DBD). The mutations are not evenly distributed, and certain missense mutations occur more frequently than others in human cancers. Indeed, 10 hotspot mutations account for ~50% of the mutations found in p53 (*4*, *5*).

The p53 protein functions as a tetramer (*6*) binding to specific sites in chromosomal DNA and activating transcription of a large set of genes, including those that can induce apoptosis, cell cycle arrest, cellular senescence, and metabolic changes. The protein is normally at very low abundance and is induced by DNA damage and other stress signals. Initially, mutant p53 proteins were found to act as oncoproteins in cell transformation assays. It was established that this effect was due to DNEs whereby mutant p53 inhibited wild-type p53 transcription factor function (*7*) by forming a mixed tetramer. Boettcher *et al.* examined this DNE extensively in a cell growth model of myeloid cancer. They compared all 7860 possible mutations in p53 for their potency at inhibiting wild-type p53. The most effective mutations were exactly the same as the mutations found to predominate in myeloid malignancies from a cohort of 1040 patients. This implies that selection for DNEs is the key driver behind the mutational spectrum found in human myeloid cancers.

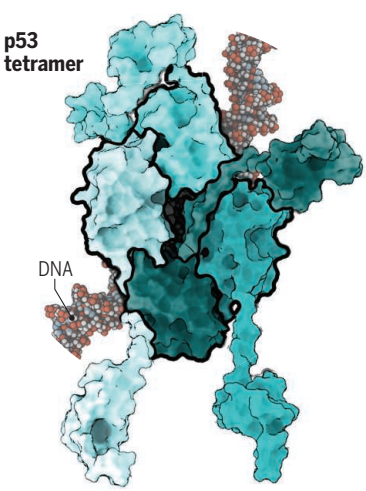
Although these data support the importance of DNEs by p53 mutation, it should not be overinterpreted. Gene dosage effects are important at the *TP53* locus. In keeping with its role as a tumor suppressor gene, loss of p53 is sufficient to increase cancer incidence. In the human Li-Fraumeni syndrome, germline inheritance of a null mutant *TP53* allele results in a wide spectrum of early-onset cancers, including osteosarcomas in young children and breast cancers in young women. If the DNEs were highly penetrant, then Li-Fraumeni patients carrying particular DNE point mutations would be predicted to have a worse prognosis than those that inherited null *TP53* mutations that prevent any p53 protein being produced. Indeed, when these cohorts are compared, only rather subtle distinctions can be made (*8*).

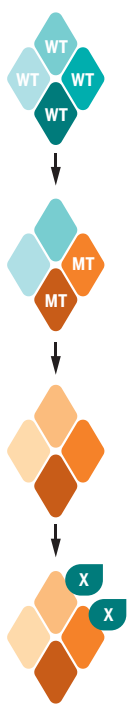
Similarly, in mice in which p53 is ablated, cancer develops within the first few months of life. In mice that carry one wild-type allele and one null allele, responses to ionizing radiation (which induces DNA damage) are reduced. In analysis of tumors arising in mice with a heterozygous genotype, loss of the wild-type allele was found not to be necessary for the formation of tumors (*9*). Two classes of tumors could be distinguished: those that retained the wild-type allele were genetically stable and still able to show p53-dependent transcriptional responses, and those in which the wild-type *TP53* allele had been lost were genetically unstable and transcriptionally inactive. Thus, reduction, rather than complete loss, of p53 activity is sufficient to increase the incidence of cancer, and cancers can grow that retain wild-type p53 activity, perhaps at a reduced rate (*9*).

Therefore, the DNE may be affected by the amount of p53 protein being expressed. In normal cells, p53 expression is exceptionally low as the protein is continually marked for degradation by the ubiquitin ligase MDM2. In the study of Boettcher *et al.*, the myeloid leukemia cells were treated with either chemotherapy or specific MDM2 inhibitors to demonstrate the DNE. These treatments

Effects of p53 mutations

The tumor suppressor p53 is commonly mutated in various cancers. It is thought that these mutations may not simply cause a loss of function (LOF), but that they may also have dominant-negative effects (DNEs) on wild-type p53 and possibly confer gain of function (GOF) that promote tumorigenesis.





Wild-type p53
Wild-type (WT) p53 is a tetrameric transcription factor.

DNE mutant/ wild type
Mutation in one allele can cause DNEs in which the mutant (MT) dimer poisons the wild-type dimer.

p53 LOF mutant
An all-mutant tetramer may have LOF of wild-type activity.

p53 GOF mutant
All-mutant tetramers may show GOF through new protein interactions with protein X.

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raise the concentration of p53 in the cells, and this is important because p53 is assembled as dimers during translation at the polysome (mRNA-ribosome complex) (*10*), and then the dimers form tetramers. Thus, in a cell expressing a wild-type allele and a missense mutant allele, depending on the concentrations of the proteins, more (or fewer) tetramers may be formed in the cytoplasm. Dimers entering the nucleus will engage with p53 binding sites, and no DNE will be seen. However, at higher concentrations, mixed tetramers would form in the cytoplasm, inactivating three-quarters of the total p53 in the cell. This implies that mutant p53 acts as an inducible dominant-negative protein and helps to explain the variation seen between biochemical and genetic analysis (*11*).

Extensive studies have implied that the mutant p53 proteins may act directly as oncoproteins not only through DNEs on wild-type p53 but also through GOF, such as the interaction with new protein targets, and that this contributes greatly to the importance of p53 in human cancer (see the figure). Such neomorphic functions would represent important targets for cancer therapy (*12*). These studies are based on a number of different experimental approaches, but perhaps the most compelling are mouse studies in which the mutant p53 protein is genetically deleted from growing tumor cells, resulting in inhibition of tumor growth (*12*). In the study of Boettcher *et al.*, no evidence for GOF is seen either in cell-based assays or in analysis of clinical samples. Reconciling these different findings is not straightforward but strongly implies that GOF must exert itself specifically in epithelial malignancies (and not myeloid malignancies), consistent with its role in promoting invasion and metastasis. Because the mutational spectra appear similar between myeloid and epithelial malignancies, the biochemical properties required for DNEs must also be exactly required for GOF, perhaps implying that the GOF target(s) is in some way biochemically related to p53 itself. ■

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NEURODEGENERATION

Early network dysfunction in Alzheimer's disease

Small aggregates of β-amyloid peptide can hyperactivate neurons, which compromises neural networks

By **Dennis J. Selkoe**

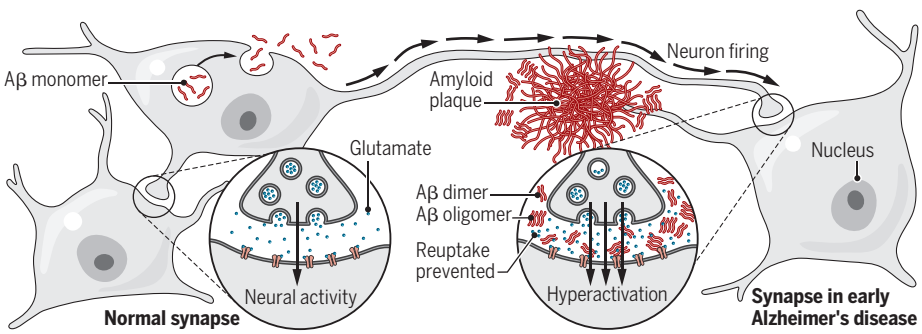
Aggregation of the β-amyloid peptide (Aβ) in brain regions serving memory and cognition is thought to initiate Alzheimer's disease (AD) (*1*). Despite myriad studies of the neurobiological effects of the peptide, two central questions remain unsettled: What forms of Aβ are the principal bioactive neurotoxins in humans, and precisely how do these forms undermine neuronal function? Attempts to therapeutically lower or neutralize Aβ in humans have so far failed to definitively slow AD symptoms, and successful approaches may require answers to these two queries. On page 559 of this issue, Zott *et al.* (*2*) provide compelling evidence that the first answer is soluble Aβ dimers, and the second answer is through hyperexcitability of glutamatergic neurons when the dimers interfere with the reuptake of extracellular glutamate.

Among the many complex facets of AD pathogenesis, selective neuronal hyperactivity has recently emerged as a particularly noxious process for the finely tuned circuit functions that underlie cognition (*3*). Studies in both the laboratory and the clinic increasingly support the pathogenic importance of abnormal neuronal activation (*4*), including the occurrence of epileptic seizures in some patients with AD (*5*, *6*). Functional magnetic resonance imaging (fMRI) of humans with prodromal (early symptomatic) AD has demonstrated excessive neuronal activity in the hippocampus and neocortex, where Aβ accumulates in abundance (*7*). The molecular basis of this hyperexcitability has been examined in transgenic mouse models of AD-like Aβ deposition and suggests a key role for soluble forms of Aβ (*8*, *9*), which are in a complex equilibrium with deposits of insoluble Aβ fibrils (amyloid plaques).

Zott *et al.* used two-photon imaging to quantify calcium and glutamate transients as a measure of the activities of individual hippocampal neurons in the living mouse brain—both in wild-type mice and a transgenic mouse model of AD. These elegant in vivo experiments were paralleled by confirmatory analyses of neuronal hyperactivity in mouse hippocampal slices in vitro. Both approaches demonstrated the absolute requirement for baseline glutamatergic neuronal firing to allow subsequent hyperactivation when soluble human Aβ dimers (and oligomers thereof) were infused into the hippocampus. Mechanistically similar hyperactivation was achieved by infusing a glutamate reuptake blocker (TBOA). The authors propose a "vicious cycle" of Aβ-mediated hyperactivation in the brain: Baseline firing activity of glutamatergic synapses allows low-nanomolar concentrations of soluble Aβ dimers (which they isolated from the neocortex of patients who died with AD) to block the reuptake of synaptically released gluta-

A vicious cycle of neuronal hyperactivation

Zott *et al.* show that soluble β-amyloid (Aβ) dimers and oligomers, which are formed during Aβ aggregation, are neurotoxic. They preferentially affect repeatedly activated neurons by blocking glutamate reuptake by neurons and astrocytes. This leads to hyperactivation and eventually neurodegeneration.



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