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Gain-of-Function Variomics and Multi-omics Network Biology for Precision Medicine

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Abstract

Traditionally, disease causal mutations were thought to disrupt gene function. However, it becomes more clear that many deleterious mutations could exhibit a “gain-of-function” (GOF) behavior. Systematic investigation of such mutations has been lacking and largely overlooked. Advances in next-generation sequencing have identified thousands of genomic variants that perturb the normal functions of proteins, further contributing to diverse phenotypic consequences in disease. Elucidating the functional pathways rewired by GOF mutations will be crucial for prioritizing disease-causing variants and their resultant therapeutic liabilities. In distinct cell types (with varying genotypes), precise signal transduction controls cell decision, including gene regulation and phenotypic output. When signal transduction goes awry due to GOF mutations, it would give rise to various disease types. Quantitative and molecular understanding of network perturbations by GOF mutations may provide explanations for ‘missing heritability” in previous genome-wide association studies. We envision that it will be instrumental to push current paradigm toward a thorough functional and quantitative modeling of all GOF mutations and their mechanistic molecular events involved in disease development and progression. Many fundamental questions pertaining to genotype–phenotype relationships remain unresolved. For example, which GOF mutations are key for gene regulation and cellular decisions? What are the GOF mechanisms at various regulation levels? How do interaction networks undergo rewiring upon GOF mutations? Is it possible to leverage GOF mutations to reprogram signal transduction in cells, aiming to cure disease? To begin to address these questions, we will cover a wide range of topics regarding GOF disease mutations and their characterization by multi-omic networks. We highlight the fundamental function of GOF mutations and discuss the potential mechanistic effects in the context of signaling networks. We also discuss advances in bioinformatic and computational resources, which will dramatically help with studies on the functional and phenotypic consequences of GOF mutations.

Keywords

Gain-of-Function mutations; Protein-protein interactions; Computational mutation annotation; Coding and noncoding variants; Post-translational regulation; Liquid-liquid phase separation

1 Gain-of-Function Mutations in Cancer

How variants of a genomic sequence alter biological functions and molecular activities has long been a key question of molecular biology [1, 2]. Past research in genomics has emphasized the importance of loss-of-function (LOF) and gain-of-function (GOF) mutations in understanding mutational effects on gene expression, protein activity, and phenotypic plasticity [3]. For instance, in protein-coding genes, LOF mutations disrupt or reduce protein functions compared to their wild-type counterparts [4]. On the other hand, GOF mutations produce mutant proteins that exhibit either new or overactive functions that would not usually exist [4, 5].

The field of oncology has continuously focused on identifying LOF and GOF mutations that may lead to many different types of cancers. Significantly, these mutations are usually identified in cancer patients, reflecting their clinical relevance. LOF mutations, usually involving tumor suppressor genes, can cause loss of protein function essential for inhibiting cancer-causing factors. These proteins typically suppressing cell growth or promoting cell death are now unable to function at the level they normally would, thereby promoting cancer growth. Meanwhile, GOF mutations in proto-oncogenes have the capacity to become neomorphic [4, 6], or produce new protein functions, and create other changes to cellular systems that favor overactive, uncontrolled cell growth. Compared to research on LOF cancer mutations, research regarding GOF mutations on cancer is largely limited as LOF mutations are more easily identifiable [7].This review will predominantly serve to inform on recent research advances highlighting GOF mutations in cancer genomics with a focus on covering this gap in knowledge. Meanwhile, GOF mutant effects in neurodegenerative diseases will be briefly discussed as well.

While all generally serve the same effect, GOF mutations often come in different forms. Such mutations may be categorized into two main areas of interest: protein-coding mutations and noncoding mutations. The majority of GOF mutations studied are limited to the coding regions of the genome and directly affect the makeup of proteins. The mutant proteins may further impact biological features, such as protein–protein interactions and enzymatic activity, to affect cell growth or other influences on the phenotype. Furthermore, recent literature has brought attention to significant GOF mutation changes in epigenetics and noncoding regions of the genome affecting tumor growth as well. These notable mutations affect cancer cell hallmarks through a variety of distinct molecular mechanisms. Identifying all types of mutations implicated in cancer progression is imperative for developing new ways of early detection and therapeutic interventions in cancer. In this review, the latest functional implications of both coding and noncoding mutations in cancer will be highlighted.

2 Epigenetic Regulation

The relationship between cancer-associated GOF mutations and epigenetic regulation has not been thoroughly studied, introducing a rather unexplored branch in cancer research. Without affecting DNA sequences, epigenetic GOF changes may create tumorigenic activity by influencing the physical structure of DNA or associated co-factors/modifiers instead (Fig. 1a). These changes may be induced by a diverse range of signals yet all similarly function to activate oncogenic transcriptional programs.

2.1 Histone Acetylation and Methylation

A major area of research on epigenetic regulation looks at the GOF changes toward histone acetylation and methylation. These biological processes are catalyzed by three subcategories of tools: writers, readers, and erasers. Writers are enzymes capable of modifying base pairs in DNA and histone proteins, erasers are enzymes capable of reversing the work of writers, and readers are protein domains capable of identifying epigenetic changes and mediating them [8]. Catalyzed by the writer, histone acetyltransferase, histone acetylation loosens the interaction of DNA with histones and subsequently increases transcriptional activity. On the other hand, histone methylation, carried out by the writer histone methyltransferases, can either repress or increase transcriptional activity depending on where in the histone they methylate (Fig. 1b).

Research has revealed the role of GOF mutations in histone methyltransferase and histone acetyltransferase genes, modifying their binding potential to histone tails and changing levels of methylation and acetylation. For example, GOF variants in G9a histone methyltransferase were found to increase WNT signaling through the inhibition of DKK1, implicating it as a possible cause for melanoma [9]. GOF mutations in CREB-binding protein and p300, two homologous lysine acetyltransferases, were also found responsible for amplifying DNA replication through multiple non-transcriptional and transcriptional processes [10]. Interestingly, another study discovered mutant-enhanced self-association of a histone acetylation reader, leading to increased chromatin occupancy and gene activation contributing toward pediatric kidney cancer [11]. Several studies have also found GOF mutations in PRC2, affecting a complex which methylates histone 3 on lysine 27 [12, 13]. Such mutations interfering with the role and function of PR2C have been implicated in multiple cancers, such as myeloid malignancies and malignant peripheral nerve sheath tumors among many more aggressive cancers [14, 15]. Another study found histone deacetylase inhibition to reduce GOF effects of p53, highlighting how an eraser too may be implicated in tumorigenesis [16]. A separate finding determined a GOF mutation in IDH1 to depend heavily on HDAC in order to promote glioma [17].

A GOF mutation (E1099K) in the NSD2 histone methyltransferase specific for histone 3 lysine 36 (H3K36), results in altered enzyme substrate binding and increased level of H3K36 dimethylation (transcription activation mark) and decreased level of H3K27 trimethylation (repressive mark) specifically at H3.1. H3K36me2 is normally found at the 5′ end of genes near transcriptional start sites, and mutant NSD2 plays an important role in proliferation and progression of acute lymphocytic leukemia. Further, in vivo studies suggest that the mice which are xenografted with $NSD2^{WT/E1099K}$ survive shorter in comparison to

mice xenografted with wild-type *NSD2* [18]. Chromosomal translocation is another example of GOF events which occur in 10–20% multiple myeloma cases and places *NSD2* under the control of immunoglobulin heavy chain promoter [19]. This results in overexpression of *NSD* which leads to an increase in H3K36me2 and a reduced H3K27me2 mark. The increased H3K36me2 level results in aberrant gene activation, promoting the cancer cell growth and tumor progression [20–22]. Additionally, Y641 mutation in *EZH2* increases the level of H3K27me3 and reduces the level of H3K27 monomethylation and dimethylation in B-cell lymphomas, follicular lymphomas, and metastatic skin melanoma. The cells with GOF mutation Y641 undergo enhanced motility and significant growth advantage compared to wild-type cells [23–25].

Together, these mutations reflect important areas of consideration for drug targeting. Herein lies evidence of epigenetic GOF changes promoting tumorigenesis. Showcasing the importance of uncovering GOF effects in histone modifying proteins, these examples highlight a significant area of interest for future research in cancer biology.

3 Transcription Factors

GOF mutations can also influence transcription factor binding sites as another way outside of coding regions of the genome to produce oncogenic effects. Recent research highlights tumor suppressor gene *TP53* for exhibiting multiple GOF mutant properties, including those affecting transcription factors. In recognizing genes encoding cell cycle-dependent proteins cyclin A and CHK1, GOF p53 mutants are able to localize to their regulatory regions, accelerating their transcription by inducing origin firing, protecting replication forks, and promoting micronuclei formation [26]. Other research highlights separate novel mutations affecting transcriptional control through neomorphic, GOF transcription factor interactions. A mutation resulting in an N-terminally truncated variant of a transcription factor named C/EBPα, or p30, was discovered to be implicated in leukemogenesis by binding to upstream enhancers of *NT5E*. *NT5E* regulates the expression of *CD73*, promoting cell proliferation and stopping apoptosis in leukemia cells [27]. Taken together, changes to and interactions among transcription factors, impacting noncoding regions of the genome, are shown to play a significant role in gene expression.

4 Noncoding Elements

Elements of the noncoding genome, such as promoters and enhancers, also play important roles in oncogenesis and cancer progression.

4.1 Enhancers and Promoters

The role of enhancers in gene regulation came into picture in early 80s, before that binding of regulatory proteins with DNA was known as only a factor involved in gene regulation. Genome-wide association studies show that, many mutations in cis-regulatory regions are also associated with disease (PMID: 25261935). A single nucleotide variation (SNV) in the α-globin cluster creates an entirely new promoter region which causes decreased α-globin expression leading to α-thalassaemia. The hinderance in gene expression is caused by the newly formed regulatory region which lies in between the α-globin gene and their associated

super enhancers in an orientation dependent manner (PMID: 34155213). Another study shows that the mutation in the promoter region of the telomerase reverse transcriptase (TERT), encoding a catalytic subunit of the telomerase, as the most abundant (71%) type of mutations occurring in melanomas examined in the study [30]. This mutation, when studied with reporter assays, exhibited upregulated transcription of TERT (PMID: 23348506). Genome-wide analysis of mutations in noncoding regions showed that mutations in regulatory regions such as promoters and 5' UTR are more frequent than mutations in 3' UTR and distal enhancer regions. The study reported that these mutations are recurrent in the promoter regions of PLEKHS1, WDR74 and SDHD as well as previously reported TERT which are further associated with different types of cancers (PMID: 25261935).

5 Protein–Protein Interactions

Protein–protein interactions (PPIs) are characterized by the physical association of proteins to mediate cellular processes and are also influenced by GOF mutations. Depending on the protein functions and the type of interactions, such acquired interactions may produce oncogenic effects in several unique ways [32].

Recent discoveries have shown various new GOF PPIs as a result of mutant protein p53. One mechanism in which p53 was shown to be implicated in cancer progression was through facilitating DNA replication. A recent study investigating GOF effects in mutant protein p53 found that p53 has the capacity to recruit MCM and PARP1 proteins on replicating DNA to promote replication upon DNA damage. This, in turn, causes tumorigenesis by allowing uncontrolled replication [33]. p53 was also discovered to mediate other PPIs enabling cancer progression as well. p53 mutants enhancing STAT3 activation by binding to STAT3 and displacing SHP2 were found to promote the growth of tumor cells in colorectal cancer [34]. Furthermore, another recent study elucidated the role of mutant p53 PPIs in suppressing the immune system to promote cancer cell proliferation. According to the study, p53 is able to bind to TANK-binding protein kinase 1, preventing the formation of a protein complex required for the activation of the innate immune response [35]. Taken together, these recent studies reveal a large number of neomorphic gain-of-PPI mutations in one gene alone. Further studies will elucidate the diverse role of p53 in creating neomorphic, oncogenic PPIs [36].

6 Post-translational Regulation

Enzymatic activity takes on a critical role in the activation and inactivation of proteins. This becomes immediately apparent when reviewing the significance of signaling pathways and kinase activation on cancer. Kinase activation works by a phosphorylation cascade creating eventual cellular response through a chain of events. GOF mutations can play a role in changing these pathways, creating phenotypic behaviors through altered steps in the chain of reactions.

Notably, tyrosine kinases are important for a cell to grow, move, differentiate, and undergo metabolism [37]. As such, changes to tyrosine kinases are frequent triggers for the onset of cancer, proving to be an active area of GOF research. Focal adhesion kinase (FAK) is

a cytoplasmic non-receptor tyrosine kinase that has been linked to driving invasion and metastasis in many cancer types. A recent study interrogated a GOF mutation in the GTPase RHOA and its biochemical relation toward inducing the activity of FAK and promoting diffuse gastric cancer [38]. Other GOF mutations promote separate kinase pathways, some of which related to FAK, such as one involving mutant PI3K, a kinase involved in the PI3K/AKT/mTOR pathway which is important in cell growth and proliferation. Another study determined that *WWP1* was found to inactivate *PTEN*, a tumor suppressor gene, and contribute toward overactive PI3K signaling, causing heightened cell growth [39]. A different study interrogated the ineffectiveness of a HER2 tyrosine kinase inhibitor due to high HER2/HER3 heterodimer catalytic activity. It was shown that high HER2/HER3 heterodimer activity leads to strong activation of the PI3K/AKT/mTOR pathway and eventually promotes cell growth, invasiveness, and drug resistance [40]. As demonstrated by these recent discoveries, multiple modes of action may be at play upon hyperactivation of tyrosine kinase signaling pathways.

Other work has recently explored areas of inhibiting oncogenic KRAS involved in an effector pathway which promotes cell survival and proliferation. KRAS mutants are implicated in approximately 30% of cancers. A frequent GOF *KRAS* mutation in lung cancer impairs the intrinsic GTPase function of KRAS, causing constitutive downstream signaling of multiple kinase pathways [41, 42]. Efforts in the past three decades have been made to develop therapies for patients carrying *KRAS* mutations but to no avail [43]. However, new research highlights the significant discovery of the KRAS-G12C variant with a druggable pocket. Current research is evaluating inhibitors targeting this variant for treatment of non-small cell lung cancer [44].

A majority of other oncogenic GOF mutations described in current literature further investigates other kinase pathways, high-lighting the significance toward understanding GOF kinase activity for drug targeting. GOF mutations in the *RET* (rearranged during transfection) gene which encodes a receptor tyrosine kinase important for several physiological functions drive tumor growth and proliferation in several types of cancers [45]. GOF mutations in BRAF, one of the most frequently found oncogenes in multiple cancer types, lead to overactivation of the MEK/ERK signaling pathway, promoting metastasis [46]. GOF mutations in *JAK2, CALR*, and *MPL* constitutively activate the JAK/STAT pathway, giving rise to myeloproliferative neoplasms, polycythemia vera, essential thrombocytosis, and primary myelofibrosis from pluripotent hematopoietic stem cells [47–49]. GOF mutations constitutively activating RAS and RAC1 proteins involved in the Ras signal transduction pathway promote cell proliferation, giving rise to cancers such as juvenile myelomonocytic leukemia and malignant melanoma [50–52]. GOF *KIT* mutations lead to kinase activation found in systemic mastocytosis, which is a myeloproliferative neoplasm that expands abnormal mast cells in many types of tissues. For example, gastrointestinal stromal tumors were demonstrated to have GOF mutations in *KIT*, exemplifying the apparent role of KIT in promoting cancer [53, 54]. Altogether, these diverse and abundant kinase GOF mutations showcase the wide array of kinase pathways which may be implicated in causing cancer. As such, further study on GOF mutations in proteins affecting kinase activity is significant and holds promising potential for innovative drug discovery.

7 Gain-of-Function Mutations in Other Diseases

Study of GOF mutations is not just limited to understanding cancer, but also to the development of other diseases as well. Notably, recent research has also highlighted neurodegenerative and inflammatory diseases influenced by GOF mutations, although not as well studied as in cancer.

7.1 Neurological Diseases

Neurodegenerative diseases, affecting nerve cells that cause debilitation, have been shown to be influenced in part by GOF mutations. GOF in kinase activity has been identified to contribute toward neurodegeneration. A recent study related GOF activity in protein kinase C (PKC) isozymes to unique neurodegenerative diseases. GOF mutations in PKCα were linked to Alzheimer's disease, and GOF mutations in PKCγ were linked to spinocerebellar ataxia type 14 [55]. Another study related GOF mutations in pro-inflammatory gene *TBK1*, or TANK binding kinase 1, to an increased risk in developing normal tension glaucoma [56]. Apart from kinase activity, recent study on Huntington's disease has shown that expanded CAG repeats may produce toxic truncated polyQ-containing huntingtin proteins, illuminating a new GOF mechanism [57]. Further, mutations in several different kinds of ion channels have been reported to be associated with neurodevelopmental, neurological, and/or psychiatric disorders. The patients with GOF mutation in KCNA2, which encodes for the potassium channel Kv1.2 exhibit severe phenotypic conditions in terms of epilepsy, ataxia, and intellectual disability (PMID: 25751627). Cav1.2 and Cav1.3 are two isoforms encoding calcium channels present in the brain. Mutations in these lead to several neurological abnormalities. A GOF mutation in Cav1.2 causes a rare multiorgan disorder Timothy syndrome and autism (PMID: 15454078). On the other hand, a GOF mutation in CACNA1D forming α1-subunit of Cav1.3 causes aldosteronism with seizures, neurologic abnormalities, and intellectual disability and might be the cause of autism spectrum disorder (ASD) in the patients (PMID: 25620733). In their next study, they found that the GOF mutation in CACNA1D is recurrent in patients and epilepsy (PMID: 28472301). Further, GOF mutations in CACNA1G, CACNA1H and CACNA1I encoding different subunits of Cav3 calcium channel, are associated with cerebellar atrophy, primary aldosteronism and epilepsy, respectively (PMIDs: 24277868; 17397049; 33704440). A GOF mutation in a Transient receptor potential gene, TRPA1 leads to a neuropathy disorder known as familial episodic pain syndrome (PMID: 20547126).

Current efforts at therapeutic strategies look to lowering huntingtin levels in order to counteract such GOF mechanisms [58]. As new research continues to investigate the significance of GOF effects in neurodegenerative diseases, they prove to be an invaluable endeavor for more effective therapeutics.

7.2 Inflammatory Diseases

Inflammatory diseases, characterized by harm from one's own over-inflammatory immune system, have also been linked to GOF mutations. Cryopyrin-associated periodic syndrome, an autoinflammatory disorder characterized by inflammation throughout multiple bodily tissues, was recently linked to GOF in *NLRP3*, resulting in an over-release of inflammatory

cytokines [59]. *NLRP3* GOF has also been implicated in contributing toward several other inflammatory and autoimmune diseases such as inflammatory bowel disease [60]. Another autoinflammatory disease, familial Mediterranean fever (FMF), was recently linked to GOF mutations in the *MEFV* gene which encodes pyrin, a protein involved in regulating the innate immune system [61]. Also notable, GOF mutations in the cGAS-STING signaling pathway, key to inflammation in infection, cellular stress, and tissue damage, has been shown to contribute toward a severe pediatric autoinflammatory syndrome called STING-associated vasculopathy (SAVI) [62]. GOF mutations in STING, a signaling molecule in cytosolic DNA sensing pathway, is a novel cause of familial chilblain lupus, which is a monogenic form of cutaneous lupus erythematosus (PMID: 27566796). Together, GOF mutations play a significant role in dysregulating the immune system as well as in inflammatory disease pathogenesis.

8 Protein Aggregation and Liquid–Liquid Phase Separation

Protein misfolding and aggregation lead to severe human diseases, such as Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis. However, not only the neurodegenerative diseases but also cancer has been recently shown to be affected by aggregation, particularly by the aggregation of mutant variants of the tumor suppressor protein p53, which are present in more than 50% of malignant tumors. Mostly the effective therapeutic strategies for all these diseases are focused on the prevention of these aggregation processes. The aggregation of large amorphous or amyloid fibrils terminates a pathway and includes the formation of intermediate oligomeric species and protofibrils. Prior to the formation of more stable aggregate species, several proteins involved in neurodegenerative diseases tend to undergo phase separation and form biomolecular condensates usually by transiting from liquid-like to gel-like and solid-like states. This transition is particularly applicable for several nuclear proteins that associate with RNAs and DNAs to generate membraneless organelles, such as the nucleolus.

Mutant p53 tends to form aggregates with amyloid properties, especially amyloid oligomers inside the nucleus, which are believed to cause oncogenic GOFs. DNA-binding domain of p53 (p53C) undergoes liquid–liquid phase separation on the pathway to aggregation under various conditions. Similarly, mutant p53C (M237I and R249S) undergoes phase separation, evolved to solid-like biomolecular condensates than that in the case of wild-type p53C (PMID: 34163823). Live cell microscopic data indicated that transfection of mutant full-length p53 into the cells results in phase separation in the nuclear compartments, which is likely the cause of the GOF effects (Fig. 2).

Considering phase separated conformers as a crucial missing link of many fundamental biological processes, these transient biomolecular condensates may be assigned to have their own function to contribute. Just like the previous example, there are a few examples on involvement of mutant proteins in different phase separated condensates. p62 assembles into condensates together with mutant KEAP1 proteins and the transcription factor NRF2, thereby affecting NRF2-driven transcription (PMID: 30126895). Although not determined, it is speculated that p62 condensates are involved in the formation and autophagy-mediated disposal of various cellular condensates that promote or inhibit tumorigenesis. Stress granule

(SG) formation was found to be induced in cancerous cells expressing mutant *KRAS*, and this was shown to be dependent on the secretion of a lipid that inactivates the eukaryotic initiation factor eIF4A (PMID: 27984728). Thus, mutant *KRAS* establishes a more stress-resistant cellular condition through SG assembly, and this confers a fitness advantage to cancer cells and presumably also resistance to chemotherapeutics. Taken together, more systematic profiling of GOF mutants in a large set of disease-associated genes is needed to correlate the current theme based on phase separation and protein aggregation.

9 Computational Resources for Predicting GOF Mutations (See Table 1)

10 Conclusion

Together, this review leads to an emerging area in molecular biology and is becoming an important area of research in the future. The area is innovative because it will provide unique insights in prioritizing functional GOF disease mutations and uncovering individualized molecular mechanisms. It is also significant because it will greatly facilitate the functional annotation of a large number of GOF mutations, providing a fundamental link between genotype and phenotype in human disease.

Since the introduction of next-generation sequencing methods, genomics research has taken a large step forward and revealed the importance of GOF mutations in contributing toward disease. Increasingly, more GOF mutations affecting cancer in the coding regions of the genome, changing PPIs and enzymatic activity, have been identified. Additionally, other novel GOF mutations in the noncoding regions of the genome, affecting transcriptional control, have also increasingly been identified and explored. Continuing forward, these GOF mutations prove to be important toward understanding both the pathogenesis of cancer and other (e.g., neurodegenerative and inflammatory) diseases. Furthermore, genomics study of GOF mutations helps elucidate potential targets for drug therapy, aiding in the combat against lethal diseases. Looking ahead, further research in GOF mutations seems promising and may bring large potential for effective treatment of various lethal diseases.

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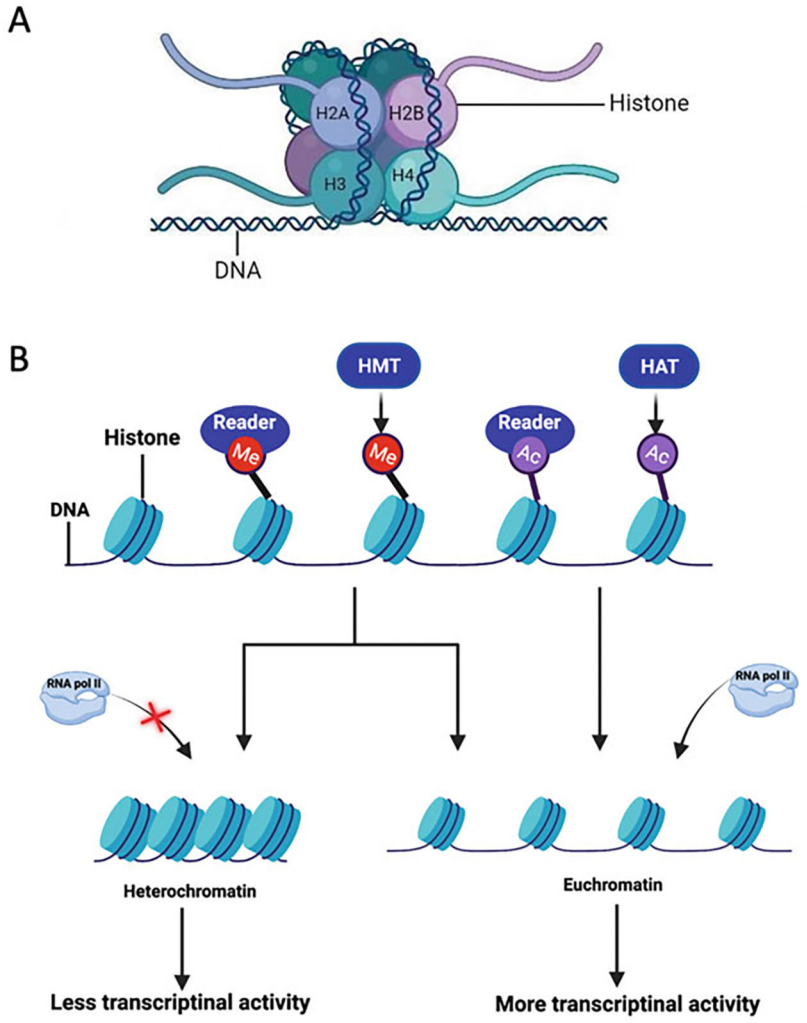

Fig. 1.

(**a**) Nucleosome consisting of histones H2A, H2B, H3, and H4 wrapped by DNA; (**b**) process of epigenetic regulation via histone acetylation and methylation. Histone acetyltransferases (HATs) act by acetylating histones, causing them to lose positive charge which loosens their interaction with negatively charged DNA. This enables RNA polymerase to better access to DNA, enabling more transcriptional activity. Histone methylation may either increase or decrease the association of histones and DNA. Depending on context, histone methylation may therefore increase or decrease transcriptional activity

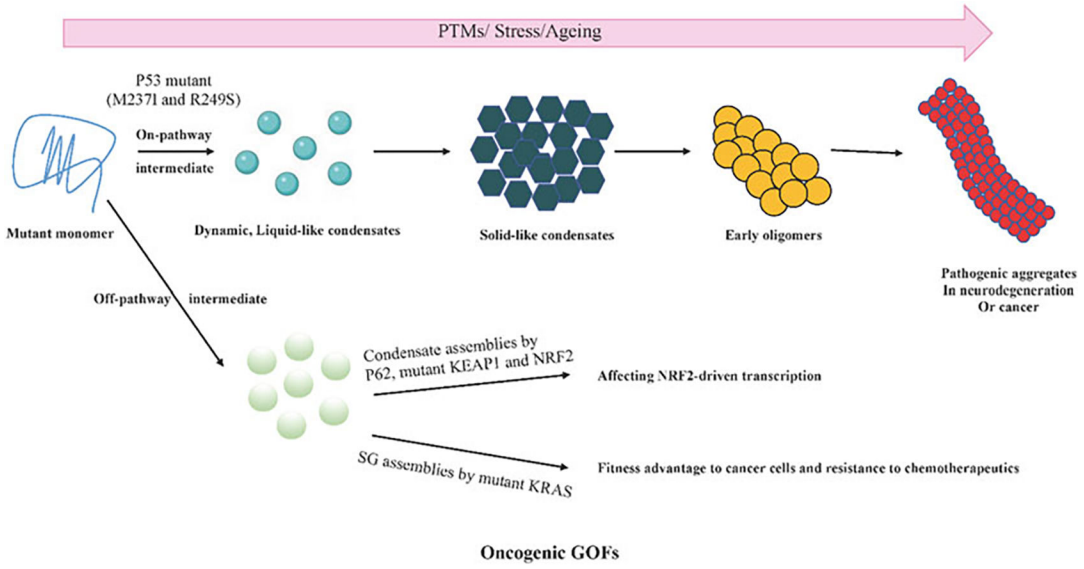

Fig. 2.

(*Scenario 1*) Mutant p53 (M237I and R249S) forms on-pathway liquid–liquid phase separation, followed by solid-like condensates which eventually leads to formation of pathological aggregates, an example of direct oncogenic GOF. (*Scenario 2*) Mutant KEAP1 assembles with p62 and NRF2 into off-pathway phase-separated condensates, which affects the function of NRF2 driven transcription. Cancer cells expressing mutant KRAS induce the formation of SG assembly which confers fitness advantage to the cells. Here both mutants are involved in phase separated assembly to accomplish an off-pathway function. So, those can be considered as example of indirect GOFs

Table 1

Computational prediction tools for GOF mutations.

Tools	Description	Web	Ref
OncoLnc	Explores survival correlations using clinical data and expression data for mRNAs, miRNAs, or lncRNAs	http://www.oncolnc.org/	[63]
OncodriveC LUST	Identifies genes with a bias toward mutations which cluster within a protein sequence	http://bg.upf.edu/oncodriveclust	[64]
Kin-driver	Human protein kinase database for driver mutations	http://kin-driver.leloir.org.ar/	[65]
KinMutRF	Identifies pathogenic variants in human kinases	https://github.com/Rbbt-Workflows/KinMut2	[66]
OncoVar	Database and analysis platform for oncogenic driver mutations	https://oncovar.org/	[67]
OncoKB	Annotates oncogenic effects and the prognostic and predictive importance of mutations	http://oncokb.org/	[68]
CHASM	Computational method which prioritizes missense mutations likely to cause functional changes that enhance cell proliferation	https://karchinlab.org/apps/appChasm.html	[69]
MEME Suite	Portal for online analysis and identification of sequence motifs representing transcription factors	http://meme.nbcr.net/	[70]
RegulomeD B	Analyzes regulatory variants in the human genome	https://regulomedb.org/regulome-search/	[71]
VEST	Scoring tool that predicts functional significance of missense mutations based on possibility of pathogenicity	http://www.cravat.us/CRAVAT/	[72]