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Current Perspective on Dominant Negative Mutations: Trends, Scope and Relevance

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Abstract: Despite the advancements in tools and technologies implicated in identifying and characterizing novel genes, there are still a significant number of unknown function proteins. Moreover, the practices employed in order to characterize such proteins have proven to be a futile exercise so far because of many limitations associated with such traditional approaches. Dominant-negative mutations have shown great promise in this direction as the introduction of mutation in the target protein may abolish the protein function and inhibit the function of the simultaneously expressed wild-type protein. These dominant mutations have broader applications in biological processes to study various proteins in terms of their functional aspects, etiological factors, and mechanism of action, paving the way to diagnose many dreadful diseases, including cancer. Considering these facts, the current review emphasizes utilizing the full potential of such dominant-negative mutations in deciphering protein functions and their broad-spectrum applications in biology.

Keywords: dominant-negative mutation; antimorph; haploinsufficiency; functional mutation.

1. Introduction

Mendel, based on his observations with respect to flower color, provided a brief description of dominant alleles. With time, the study of genetics matured, and dominance is referred to as alleles whose phenotype is manifested in heterozygous condition. Dominant alleles can also be determined phenotypically when present along with two or more recessive alleles (e.g., R/r/r) as encountered in transgenic strains. Mutations may or may not produce deterministic phenotypic characters. Mutations are known to play a pivotal role in both normal and abnormal biological processes. These are broadly classified as (1) spontaneous mutations (2) mutations due to error-prone replication (3) errors introduced during DNA repair, and (4) induced mutations caused by mutagens [1]. For the sake of experimentation, scientists have deliberately introduced mutant sequences through DNA manipulation. Various ways have been postulated to classify mutations based on structure, function, fitness (harmful or beneficial), inheritance, and impact on the protein sequence.

Mutations can also be classified based on impact on protein function, as shown in table 1. The impact of functional mutation can be beneficial or detrimental, and loss of function can be amorphic or hypomorphic. Hypomorphs are usually recessive but can be dominant occasionally due to haploinsufficiency, where a single functional copy of a gene does not produce enough protein resulting in the abnormal or diseased state. It is responsible for some

of the autosomal dominant disorders. However, the gain of function may give rise to hypermorphic, anti morphic, and neomorphic mutations. Antimorphs are dominant-negative mutations acting antagonistically to normal gene activity. The phenotypic severity of an antimorph is worse in heterozygous conditions. The increase can reduce it in wild-type gene function [2]. The functionality of protein dimer consisting of normal and mutated protein might also be affected by anti morphic mutation (Fig. 1). Altered gene product from such mutations acts antagonistically to the wild-type protein. These mutations usually result in inactive protein showing dominant or semi-dominant phenotype. The role of dominant-negative mutations in cancer-related genes like p53 [3], ATM, CEBPA, and PPAR gamma has been found in humans [4-6]. Detail of various dominant-negative mutations in humans is compiled in the OMIM database (Table 2).

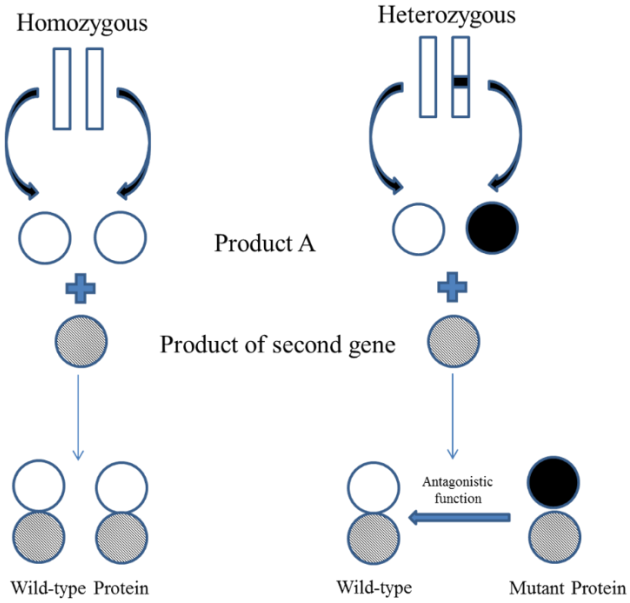

Figure 1. Role of Dominant-negative mutation on multimeric proteins.

1.1. Dominant mutations: dominant-negatives versus haploinsufficiency.

Mutations acting as dominant may not behave the same. This article focuses on two such mutations: Dominant-negative and Haploinsufficiency, and mechanistic insights on how these mutations confer a dominant phenotype. Dissimilar dominant alleles may require different mapping strategies. Different situations that could give rise to various types of dominant alleles will be discussed considering the hypothetical dap-1 gene.

2. Dominant-negative alleles

An allele can show dominant, semi-dominant, or recessive function depending on the accomplice allele and phenotype considered. For clarification, consider alleles C and D, with genotypes CC, CD, and DD. Observation of specific phenotypic characters in CC and CD genotypes contradictory from DD genotype proves that allele C is dominant to allele D. On the contrary, allele C and D are considered co-dominant if CD phenotype is in the middle or joins characters from both CC and DD phenotype [2,7].

Huntington's disease gives an uncommon example of the freak allele that is predominant to wild-type as homozygotes are less influenced than heterozygotes [8-11]. Huntington protein interacts with over 100 other proteins and appears to have multiple biological functions [12]. The behavior of this mutated protein is not completely understood.

However, it is toxic to certain cell types, particularly the brain. Early damage is most evident in the striatum. However, other areas of the brain are also more conspicuously affected by the progression of the disease. Early symptoms are attributable to the striatum functions and its cortical connections that control movement, mood, and higher cognitive function [13,14]. Huntington's disease might also involve epigenetic alterations like DNA methylation and histone modification [15,16].

2.1. Special role of dominant mutations on behalf of their functional determination potential.

Dominant-mutations can have both detrimental as well as beneficial effects on biological research. As so far, the disease-causing effects of dominant mutations have been observed, but that does not mean that these mutations cannot be used for biological research. The following section considers this point where the first part will cover such mutations' role to determine or ascertain the function of highly conserved genes with specific examples. The second part will focus on the gain of function research and the concerns behind it.

Table 1. Mutations based on protein function.

Types	**Characteristics**
Amorph	Complete loss of protein
Hypomorph	Reduction in protein function
Hypermorph	Increase in protein function
Neomorph	Development of new function
Antimorph/Dominant-negative	Mutant protein interferes with wild-type protein

Table 2. Few examples of Dominant-negative mutations in humans (OMIM Database).

OMIM Number	**Gene, Protein**	**Phenotype**
262500	*GHR*, growth hormone receptor	Laron syndrome
173110	*POU1F1*, POU domain, Class1, Transcription factor 1	Pituitary hormone deficiency
139250	*GH1*, growth hormone 1	Growth hormone deficiency
190160	*THRB*, thyroid hormone receptor β	Thyroid hormone resistance
138850	*GNRHR*, gonadotropin-releasing hormone receptor	Hypogonadotropic hypogonadism 7 without anosmia
165230	*GLI2*, GLI, Kruppel family member 2	Culler-Jones syndrome
191740	*UGT1A1*, UDP glycosyltransferase 1 superfamily	Gilbert syndrome
191170	*TP53*, tumor protein p53	Li-Fraumeni syndrome
600140	*CREBBP*, CREB- binding protein	Rubinstein-Taybi syndrome
194070	*WT1*, Wilms tumor 1	Denys-Drash syndrome

2.2. Use of dominant negatives in the determination of cellular function.

Candida albicans (*C. albicans*) cause more serious infections than any other fungus, but because of its diploid genome and lack of sexual reproduction, it becomes difficult to study this organism using classical genetics. Till now, many powerful molecular methods have been developed for studying biology and pathogenesis caused by *C. albicans*, but still, many fundamental questions cannot be concretely answered using cloning and gene disruption. Pathways for protein trafficking are highly conserved in all eukaryotes. Detailed studies have been carried out on *Saccharomyces cerevisiae* to study this secretion pathway. Dozens of *S. cerevisiae* genes encoding key components, especially those controlling the pathway mentioned above, have been cloned to analyze the details [17-19]. *C. albicans* exhibit homology with *S. cerevisiae* secretion pathway genes, hence *C. albicans* homologs (SEC18, SEC14, and SEC4) [20-22] of three essential *S. cerevisiae* secretion pathway genes have been the target of extensive study. All of these three genes complement the corresponding mutations in *S. cerevisiae*, and their deduced protein products corresponding to *S. cerevisiae* have been

found to be 50-67% identical [21]. In *S. cerevisiae*, SEC4 encodes a small Ras-like GTPase (Sec4p), which is required for the fusion of post-Golgi secretory vesicles to the plasma membrane [23]. As the SEC4 gene is essential for *S. cerevisiae* therefore, sec4 null mutants are nonviable. Dominant-negative mutations in *S. cerevisiae* SEC4 gene leads to inhibition of growth and protein secretion and cause post-Golgi secretory vesicles to accumulate intracellularly [24]. The role of the SEC4 gene in *C. albicans* was highlighted by Mao *et al.* [25] by cloning homolog of SEC4 after complementation of temperature-sensitive *S. cerevisiae* sec4 mutant and using site-directed mutagenesis to successfully construct mutant sec4 allele which was analogous to those encoding trans-dominant inhibitors of other ras-like GTPases. Their study observed that the sec4 mutant phenotype could be induced even if there was the presence of two wild-type SEC4 alleles, which indicated the role of sec4 (S28N) as a dominant-negative mutant allele. The study proved pivotal as sec4 (S28N) overexpression was not able to inhibit growth and protein secretion in a minority of galactose-incubated transformants. Secondly, phenotype exhibiting growth inhibition was lost after prolonged incubation in galactose. Their results concluded that the SEC4 gene in *C. albicans* is primarily required for proper growth and protein secretion. However, it functions at later stages in the protein secretion pathway than the formation of post-Golgi secretory vesicles [25].

2.3. Typical examples of dominant-negative mutations.

Dominant-negative effects have been related to proteins involved in signaling and transcriptional activity. A specific example is provided by Drosophila's dorsal (dl) protein's DNA binding activity, which depends on dimerization. Most mutations are true recessives, but one particular mutation exerts a dominant-negative effect. This is an Arg-*Cys substitution that maps to the DNA binding domain but does not affect oligomerization. It appears to act by abolishing the DNA binding of normal/mutant heterodimers [26]. Another example of a dominant-negative mutation in Drosophila is Abruptex (*Ax*) missense mutation at Notch locus [27,28]. Similarly, the more severe phenotype associated with Wilms tumor (WT1) mutation in Denys-Drash syndrome, as compared with Wilms's a tumor/genitourinary abnormalities, may be explained by the dominant-negative behavior of specific zinc finger mutations in the former condition. It is not yet certain whether this is mediated by WT1 dimers [29,30].

Dominant-negative mutation in KIT proto-oncogene results in pigmentation related disorder manifested as white spotting (W) in mouse [31] and piebaldism in humans [32]. KIT encodes receptor tyrosine kinase, which undergoes dimerization in response to ligand binding to pursue its activity. There can be a severe phenotype of piebaldism depending upon the type of mutation. Frameshift mutation resulted in a mild phenotype in heterozygous condition, which was supposed to be attributed to haploinsufficiency. Splice junction mutation affected intracellular tyrosine kinase domain, inhibiting signal transduction and increasing the disease [32]. The dominant-negative effect is seen as a contributory factor resulting in variable phenotype through truncations in the same domain as seen in an analogous truncation of fibroblast growth factor receptor (FGF-R) [33].

Dominant-negative effects can also be very useful in the case of neoplasia. Mutation in tumor suppressor gene (*p53*) might result in neoplastic growth [34]. Although *p53* is conventionally viewed as a "recessive" tumor suppressor gene, some mutants can deregulate p53 function in a dominant-negative fashion. A large number of p53 mutations have been described, but most of them are missense mutations concentrated in four hotspots [35]. Some of these mutations can change the conformation of p53. In *in-vitro* conditions, p53 can adopt

either active or inactive conformation; wild type protein is normally in an active state. Co-translation with certain missense mutants results in mixed oligomers that adopt the inactive conformation [36]. In contrast, no alteration in wild-type activity is induced by co-translated missense mutant associated with the Li-Fraumeni syndrome suggesting that Li-Fraumeni p53 mutants may be relatively weak. It should be noted that p53 oligomerization domain lies at the extreme C-terminus, so prematurely truncated forms cannot bind wild type and therefore cannot act in a dominant-negative fashion.

Herskowitz described two models for the dominant-negative effect [37]. Mutant protein can either show toxicity in a multimeric complex or compete with wild-type to bind the target factors. Representatives of both classes are known to be an example of the first model of negative regulation by forming inactive heterodimers of transcription factors MyoD and c-Jun by Id and JunB proteins, respectively. Id protein is a truncated helix-loop-helix (HLH) protein that forms a dimer with MyoD but lacks the basic region required for DNA binding [38]. Similarly, critical amino acid substitution in JunB results in the formation of inactive JunB/c-Jun heterodimer rather than its homo-dimerization [39]. The second model is explained by the interferon activator (IRF1) and its antagonist (IRF2). IRF2 has enhanced DNA binding potential and displaces IRF1 from the interferon promoter but acts as a weak activator [40].

3. Haploinsufficiency

This condition is where a single functional allele is not enough to provide functional protein when the other copy is compromised. This leads to loss of function and gives this mutation the name of haploinsufficiency. This situation will be clarified using hypothetical dap-1gene. Assume that a certain threshold activity of dap-1 is essential to avoid the abnormal phenotype P1. To achieve this threshold level, two copies of wild-type genes are required. Mutations occurring in the dap-1, which can reduce or eliminate its activity, would therefore behave dominantly. This is because, in the heterozygous animals, the remaining single wild-type allele of the dap-1 gene will exhibit insufficient levels to exhibit wild-type gene activity.

Hence the loss-of-function on the dap-1 mutant allele (*dap-1*) may produce a similar phenotype whether present in one or two copies, behaving dominantly. Contrarily, *dap-1*/dap-1 heterozygous animals shall exhibit a phenotype that differs quantitatively or qualitatively from homozygous *dap-1/dap-1* animals because the former case still produces half of the normal gene dose.

3.1. Haploinsufficiency as an emerging cause of diseases and their complications.

Various diseases, especially syndromes, arise and/or complicate on account of genetic defects and mutations. Dominant mutations are no exception to this, but detection of their specific role and mechanism has always been a challenge to the researchers, as seen in the case of haploinsufficiency. Haploinsufficiency has been found to be closely related to a number of diseases, including cancer, as per recent reports [41].

a) Role of haploinsufficiency in *CRIM1* gene that codes for cysteine-rich motor neuron 1 protein resulted in defects in human and mice eye development as reported by Beleggia *et al*. [42]. Some additional reports also have a related role of gut microbiota to neurological disorders and other health risks [43,44].*Colobomatous macrophthalmia* with microcornea syndrome (MACOM) is an autosomal dominant malformation of the eye characterized by (1) microcornea with increased axial length (2) coloboma of the iris and of the optic disc, and (3)

severe myopia. Role of *CRIM1* gene during eye development in mice was targeted through a cross between the $Crim1^{flox}$ mouse line and Ap2α-cre mouse line. Results showed alterations of eye development in homozygous mice resulting in severe morphological changes. These findings were synonymous with anomalies observed in MACOM patients and confirmed *CRIM1* gene as a causative agent for MACOM syndrome. This further emphasized that haploinsufficiency of *CRIM1* may cause abnormality in eye development [42].

b) Similar role of haploinsufficiency was found in the *MYBPC3* gene that codes for cardiac myosin binding protein-C. This protein is a special component of thick filaments forming striated muscles. Studies suggest that MyBP-C may have both structural and regulatory roles within the sarcomere [45,46]. The physiologically significant role of MyBP-C has also been found as mutations in *MYBPC3* were identified as one of the most common causes of hypertrophic cardiomyopathy (HCM) [47,48]. Out of these, approximately two-thirds of *MYBPC3* mutations are predicted to generate truncated protein [49,50]. Till recent times it was unpredictable whether the autosomal dominant nature of *MYBPC3* mutations results from haploinsufficiency or poison peptide effect (on the virtue of which the mutant proteins interfere with normal sarcomere function). It has always been a rationale statement to the research community that most of the sarcomere gene mutations causing HCM are the missense alleles that encode dominant negative proteins. However, mutations in *MYBPC3* exist as few exceptions because it frequently encodes truncated proteins. Marston *et al*. [51] and others [52] tried to determine the evidence of haploinsufficiency in *MYBPC3* mutations causing hypertrophic cardiomyopathy. Their study highlighted that in human myectomy samples with *MYBPC3* truncation mutations, no detectable amount of truncated MyBP-C protein was found in either incorporated or unincorporated form. Analysis of heart samples of patients bearing *MYBPC3* mutations revealed 24% lower MyBP-C content. This strongly argues for haploinsufficiency as the disease mechanism for both truncation and missense mutations similar to earlier observations [53-55]. It also argued against the incorporation of truncated MyBP-C protein to cause any dominant-negative effect due to the absence of any detectable truncated MyBP-C.

c) Haploinsufficiency event might shape the disease and complicates it to a much severe degree, as seen in the case of mucinous cystic neoplasms (MCNs). Izeradjene *et al*. [56] reported from their study that concomitant expression of $Kras^{G12D}$ and haploinsufficiency of Smad4/Dpc4 tumor suppressor gene instigate a whole new class of pancreatic tumors known by the name of mucinous cystic neoplasms (MCNs). The disease's progression was found to be accompanied by loss of heterozygosity of Dpc4 and mutation in either of the *p53* or *p16* gene [57,58].

3.2. Role of haploinsufficiency in disease modification.

a) Modification of EGFR driven tumorigenesis through Trp53 haploinsufficiency: Alteration in expression levels of *TP53* and *EGFR* genes is seen in malignant peripheral nerve sheath tumors (MPNSTs), which belongs to a class of aggressive sarcomas. In this regard, Rahrmann *et al*. [47] worked on determining co-operation between underexpression of cell cycle regulator *TP53* gene and over-expression of epidermal growth factor receptor (*EGFR*) gene for the transformation of Schwann cell *in-vitro* and formation of MPNST *in-vivo*. The collective results, which comprised human gene copy number alteration data, microarray data, and thrombotic microangiopathies (TMA) data, indicated co-occurrence of EGFR over-expression *TP53* haploinsufficiency in human MPNST samples. Simultaneously, *in-vivo*

studies on transgenic mice over-expressing *EGFR* in Schwann cells and heterozygous for *Trp53*-null allele showed a significant increase in MPNST formation.

b) Haploinsufficiency of *BRCA1*: Breast cancer has always been a challenge to cancer biology experts. The tumor suppressor gene *BRCA* is what they study extensively to find therapeutic measures against the disease. *BRCA1* and *BRCA2* genes expressed in breast and other tissue cells have physiological importance of repairing damaged DNA or destruction of affected cells if the damage is beyond repair. Mutation in either of these genes is responsible for the majority of breast cancer cases [59,60]. Numerous mutations of *BRCA1* gene have been identified, and many have shown an association with increased risk of cancer. A recent study by Feilotter *et al*. [61] related *BRCA1* haploinsufficiency with alteration in gene expression that functions in cellular proliferation and development. The researchers developed a biological assay for haploinsufficiency of *BRCA1* where they compared the expression pattern of genes in *BRCA1* wild-type cells with those carrying (heterozygous) *BRCA1* pathogenic mutations. They identified a subset of 43 genes whose combined expression pattern can be used as a predictor of BRCA1 status. This subset comprised of genes involved in cellular differentiation. Their study concluded that single copy loss of *BRCA1* function might affect differentiation making cells more susceptible to malignancy. However, *BRCA1* haploinsufficiency at lower doses may not cause DNA damage at a global level but facilitates such change at higher doses.

Consistent with the findings from other studies, down-regulation of interferon regulated genes in BRCA1+/− cells highlighted the role of *BRCA1* haploinsufficiency in the deregulation of interferon signaling [61]. Earlier studies on breast tumors of *BRCA1* carriers reported a higher rate of LOH of wild-type allele [62-64]. However, there exists a few cases where tumor tissue exhibits loss of the mutant allele and normal tissue shows LOH of the wild type *BRCA1* allele. Few studies also support the complete loss of *BRCA1* in sporadic cancer as a rare event [65,66]. However, a different mechanism might be involved in *BRCA1* inactivation in sporadic cancer, or *BRCA1*-dependent oncogenesis is associated with haploinsufficiency during initial developmental stages.

3.3. Variants of haploinsufficiency in various cancer types.

Gene over-expression or increased dosage has been commonly accepted as one of the causative events in cancer formation. Surprisingly, there exist a number of evidence for the role of haploinsufficiency in oncogenic events, but still, this concept has lagged far behind in relation to cancer. Cancer biologists consider proto-oncogenes and tumor suppressor genes (TSG) as two broad classes of high penetrance genes mutated in cancer [67-70]. Oncogenes are known to be activated by dominant mutations that enhance the function of a gene product. In contrast, tumor suppressors must be fully inactivated to initiate tumor progression. Hence, it was expected that mutations in these genes would act in a recessive manner. The concept of recessive TSG function in cancer was explained using the ‘two-hit’ hypothesis [71,72]. Due to the high probability of the second hit, it was supposed that the cancer susceptibility syndrome was inherited dominantly, but mutations in a recessive gene caused it. This informative theory led the two-hit hypothesis as the beginning of TSG identification generations such as *BRCA1*, *APC*, *BRCA2*, and *VHL* [63,73-75]. Finally, it helped identify *p53* as a tumor suppressor gene rather than an oncogene [76,77].

a) TSG haploinsufficiency in tumor formation with an example of p53: Two broad classes of TSGs have been identified as gatekeepers and caretakers. Caretaker genes (e.g. *p53*) prevent or repair the damage that occurred in the genome, thereby reducing the rate of mutation

acquisition and cancer. The gene *p53* is found to be mutated in at least 50% of human cancers and may prove higher in several cases [78-81]. Though *p53* is now considered a potent TSG it was not always the case when initial studies of *p53* suggested that it exhibit oncogenic properties [82-85]. Few published studies indicated that oncogenic properties resulted from mutations in wild-type tumor-suppressor *p53* [86-88]. Such mutations are responsible for the formation of truncated proteins resulting in deregulation of gene function. In some cases, this mutation acts as a dominant-negative for wild-type p53 protein as they stabilize the normally labile p53 protein. The *p53* locus is prone to deletion, as in human cancer, and loss of heterozygosity (LOH) appears in a few tumors docking p53 mutation [89]. The haploinsufficiency of *p53* in promoting tumorigenesis is seen as a loss of one copy of *p53* gene, which gives rise to an intermediate phenotype. Certain other genes such as *PTEN* exhibit the property of 'obligate haploinsufficiency' where heterozygous state promotes a greater degree of cancer than a homozygous state. The hint of haploinsufficiency in *p53* was found by analyzing Li–Fraumeni syndrome patients (LFS) [90]. Germ-line mutations cause the majority of LFS cases in *p53* [91,92]; generally, these are missense mutations, but deletions can also occur, resulting in truncated proteins. Study based on analysis of tumors from LFS patients focussed on LOH of *TP53* gene. Approximately 60% of the tumors analyzed revealed LOH at *p53* locus [93], highlighting that single-copy loss of *TP53* could contribute to tumorigenesis rather than the previous belief of *p53* mutations acting as dominant-negative inhibitors of the wild-type allele. To address this issue, tumors were analyzed from *p53* allele knockout mice. It was observed that homozygous p53 knockout (−/−) animals are viable but readily develop several types of tumor. Lethality appears by the age of 10 months. The survival of *p53* heterozygote (+/−) animals was intermediate to wild-type (+/+) and (−/−) animals. These heterozygous *p53* (+/−) animals develop tumors that do not exhibit loss or mutation of the remaining *p53* allele [94]. This shows that heterozygosity of *p53* is sufficient for tumorigenesis [95].

This fact was further supported by the study based on quantifying the transcriptional response to *in-vivo p53* expression using transgenic *p53*-reporter mouse strain [96]. These animals had transgene in which expression of lacZ reporter was driven by *p53*-responsive promoter 'Mdm2'. Researchers working on this model noticed that *p53* (+/−) embryos carrying this transgene exhibited severely low lacZ activity. This observation concluded that p53 levels prove to limit for transcription of target genes. Hence, *TP53* gene can be considered haploinsufficient in such cases [96].

These studies, including several others, indicate that *p53* haploinsufficiency is capable on its own to promote cancer and *TP53* expression levels prove pivotal for tumor initiation and progression.

b) Haploinsufficiency in another TSG (*PTEN*): Like *TP53* another TSG, '*PTEN*' which codes for Phosphatase and tensin homolog (PTEN) protein, is involved in suppression of numerous cancer types. *PTEN* is mutated or lost in a large fraction of human cancers, including breast cancer, endometrial cancer, prostate cancer, colon cancer, and glioblastoma [97]. Numerous studies from human and mice models provide evidence to support the role of *PTEN* haploinsufficiency in the promotion of cancer. In addition to this, *PTEN* haploinsufficiency is co-related with other genetic events to promote prostate cancer [59,98-100]. In contrast to *p53*, *PTEN* functions as gatekeeper and negatively regulates proliferation signals [101-104]. As discussed above, *PTEN* defines a case of 'obligate haploinsufficiency' where loss may give rise to gain when it comes to tumor suppression. Two independent studies suggested this that

complete loss of *PTEN* may actually be less tumorigenic than a heterozygous loss of *PTEN* [105-107].

The explanation for this seems complex but is based on multiple gene interactions. Complete loss of *PTEN* expression activates *TP53*-dependent cellular response, which in turn acts as a brake for tumor formation [105]. In contrast to this, heterozygous loss of *PTEN* can enhance proliferation and acts more as tumorigenic. Mutated *TP53* cells that are markedly unable to activate p53 do not undergo *PTEN* loss-induced cellular senescence [108], and hence complete *PTEN* loss proves more tumorigenic than the partial loss of the gene. These findings suggest that complete loss of *PTEN* cooperates with partial or complete loss of *TP53*. Thus, *PTEN* clearly validates a new theory of 'obligate haploinsufficiency'. As evident from the examples given above, the mechanism depends on the conditions, hence the theory becomes conditional.

Another complex situation is of 'compound haploinsufficiency', where haploinsufficiency of multiple genes in the region cooperates together and promotes tumorigenesis. In this case, a single gene alone is not capable of promotion or initiation of tumorigenesis, but heterozygosity of that particular gene in co-operation with another results in haploinsufficiency and cancer development. This situation can be symmetric or asymmetric. Asymmetric haploinsufficiency occurs when haploinsufficiency of one gene promotes the phenotype expressed by another gene but not vice-versa. This condition was exemplified by Ma *et al*. [109], which enlightened asymmetrical haploinsufficiency in tumor suppression because of functional interaction of *PTEN* and *Tsc2* genes in mice.

3.4. Understanding haploinsufficiency in terms of cell proliferation and regulation.

Deregulation of cell polarity proteins has always been closely associated with invasion and metastasis processes. *TRIM62* gene, also called *DEAR1* (for ductal epithelium–associated RING chromosome 1) is a regulator of cell polarity. It acts as a tumor suppressor gene, majorly in breast cancer.

a) Haploinsufficiency of *TRIM62*: Quintás-Cardama *et al*. [110] examined the role of *TRIM62* in the development of lung cancer and found that haploinsufficiency of *TRIM62* co-operatively acts with K-RasG12D mutation promoting invasiveness and disruption of 3-d morphogenesis affecting epithelial-mesenchymal transitions. They also noted that these phenotypes in tumor cell lines are reverted on the re-expression of *TRIM62*. Thus, they concluded that decreased levels of TRIM62 may play an important role in the evolution of lung cancer, as evident from their results [99].

b) *NBS1* haploinsufficiency: Hypomorphic mutations cause Nijmegen breakage syndrome (NBS), which is a rare human autosomal recessive disorder. It accounts for the virtue of mutations in *NBS1* gene that causes a defect in resection of double-strand breaks. NBS1 physiologically acts as a part of MRN complex, which is a protein complex and functions both in homologous recombination (HR) and non-homologous end-joining (NHEJ). NBS is characterized at a cellular level by chromosomal breakage and defective cell cycle checkpoints. *NBS1* null mutations exhibit early embryonic lethality in mice, whereas hypomorphic mutants of *NBS1* are viable. Cells from these mice have defective S phase and G2/M checkpoints. Polymorphisms of *NBS1* have been found associated with an increased risk of breast cancer in humans [111]. Studies have found the pivotal role of *NBS1* in the clinical outcome of breast cancer in patients, but *NBS1* haploinsufficient mouse results in rare mammary tumors. For clarification, Wan *et al*. [112] examined the role of *NBS1* in mammary tumorigenesis using

NBS1+/- mice crossed with mammary tumor-prone MMTV-neu transgenic strain. They observed an increased level of mammary tumor latency in *NBS1*+/-; neu mice compared to *NBS1*+/+; neu control animals due to increased apoptosis in early *NBS1*+/-; neu mammary tumors. However, a strong metastasis level was observed in *NBS1*+/-; neu mammary tumors, thereby exhibiting a differential gene expression profile compared to control tumors. The authors thus reported increased mammary tumor latency and metastasis due to *NBS1* haploinsufficiency.

c) Haploinsufficiency in KISS1 receptor (GPR54) and *Sam68*: Effect of haploinsufficiency to cause a delay in breast tumor initiation, progression, and lung metastasis was studied by Cho *et al*. [113], targeting KISS1 receptor (KISSR or GPR54). KISS1R on activation with Kisspeptins functions in normal physiology and pathophysiology. KISS1R signaling has been associated with the inhibition of tumor angiogenesis and metastasis, but the role of *KISS1R* haploinsufficiency in tumorigenesis was recently explored [113]. They developed PyMT/Kiss1r mice by crossing *Kiss1r* heterozygous mouse (*kiss1r*+/-) with MMTV-PyMT transgenic mouse and observed that *Kiss1r* heterozygosity in MMTV-PyMT/Kiss1r+/- mouse model attenuated breast cancer initiation, growth, latency, multiplicity, and metastasis.

An effect similar to *KISS1R* haploinsufficiency was reported by Richard *et al.* [114], where *Sam68* haploinsufficiency delayed the onset of mammary tumorigenesis and metastasis. Sam68 is an RNA-binding protein often mentioned as STAR (signal transduction activator of RNA) protein. The study proved that Sam68 might act as an *in-vivo* modulator of tyrosine kinase activity and as an effective signaling molecule required for mammary tumorigenesis and metastasis.

d) *Mtbp* haploinsufficiency and tumor metastasis promotion: Contrary to the haploinsufficiency results reported above, Iwakuma *et al*. [115] revealed that *Mtbp* haploinsufficiency promotes tumor metastasis in mice. Mdm2 acts as an inhibitor of p53, but over-expression of Mdm2 with wild-type p53 in numerous tumors suggests an alternate mechanism for loss of p53 activity. The protein MTBP (Mdm2-binding protein) inhibits self-ubiquitination of Mdm2, leading to stability of Mdm2 and degradation of p53. The workers in their study used haploinsufficiency model to address the role of MTBP in the regulation of p53 pathway. They observed that sensitization of mice for tumor development by p53 heterozygosity showed significant development of metastatic tumors in *Mtbp*+/-*p53*+/- mice compared to *p53*+/- mice. The results suggested the role of MTBP as a metastasis suppressor and as a novel therapeutic target for metastasis.

3.5. Understanding haploinsufficiency about RNA.

Haploinsufficient TSGs are generally impaired by 50% reduction in expression, whereas quasi-insufficiency (less than 20% reduction) of PTEN protein level may also contribute to cancer development [116]. Studies based on mouse models suggest that targeting *TP53* by short hairpin RNAs (shRNAs) exhibit distinct phenotypes differing from hyperplasia to malignancy. All of these depend upon a reduction in protein level [117]. So we can conclude that some TSGs, like *PTEN* are exquisitely dosage-sensitive while others like *TP53* are intermediately sensitive.

As discussed earlier, the mutual co-operation of multiple haploinsufficient genes promotes tumorigenesis giving rise to ‘compound haploinsufficiency’. A popular example demonstrating the importance of combinatorial interactions exhibiting different phenotypes is

5q deletion syndrome [118]. Studies have also shown synergistic tumor growth promotion by co-suppressing TSG genes on chromosome 8p [119]. Genes and even microRNAs (miRNAs) are found to be haploinsufficient to cause developmental abnormalities in humans [120]. Some of the genes like *DICER1* and *XPO5* are involved in miRNA biosynthesis pathway and identified as haploinsufficient tumor suppressors [121,122]. MicroRNAs are also involved in gene regulation at the post-transcriptional level through RNA-induced silencing complex (RISC) either by mediating translational inhibition or by mRNA cleavage resulting in decreased protein level [123]. This aspect has broadened the dimensions of haploinsufficiency with miRNAs' role, leading to the production of an insufficient amount of proteins.

4. Conclusions

It is indeed important to understand the etiologic and mechanistic foundation of dominant-negative mutations. Efforts are underway to understand the complex intricacies of protein-protein interaction networks and how dominant-negative mutations could play a role in delineating them. However, the designing of dominant-negative proteins is of paramount importance for enhancing the study of gene function. Many oligomeric proteins in the past have been known to handle a variety of functions simultaneously. However, their defective status sometimes may result in susceptibility to a particular disease as well. Therefore, dominant-negative mutations could be an important tool to understand the association between health and disease.

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Conflicts of Interest

The authors declare no conflict of interest.

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