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Review Article

CRISPR-Modified Neurofibromin Can Help Suppress the Leukemic PI3K Signalling Pathway

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Abstract

Leukaemia is the type of the cancer that mainly deals with the formation of the haemo-tissuesin the regions like lymphatic system and the bone marrow. Several pathways associated to theleukaemia have been found by the scientists over the globe. Interestingly, the most important and one of the critical pathways is the AKT/PI3K pathway that involves a plethora of the cytokines and the growth factors. This PI3K pathway plays a major in the biological processes like angiogenesis, apoptosis, proliferation of the cells and the signal transduction related events. But, have you ever been intrigued by the fact if this prime pathway can be regulated? If regulated, then what are those key control factors and how can we regulate it in case of blood cancer? Neurofibromin is a protein that is mainly encoded by the NF1 gene with 2818 amino acids with a molecular mass of around 250 - 280 kDa that is involved in the GAP related activities and known to negatively regulate the RAS signal transduction pathwayand this pathway plays a major role in the PI3K pathway. So, there is an urgent needed to engineer the RAS protein in order the PI3K activity. Reportedly, the mice that were mutated with the PI3K protein did not form cancers. This review emphasizes on the dominant activity of the Neurofibromin protein engineered by the CRISPR technology that can indirectly regulate the PI3K pathway.

***Keywords**: Leukaemia; Haemo-Tissues; Lymphatic; Bone Marrow; Signal Transduction;Neurofibromin*

Miraculous nature of neurofibromin

Neurofibromin is a ubiquitous protein consisting of 2818 amino acids that is encoded by the NF1 gene present on the chromosome no-17 [1]. It has a total molecular mass of around 250 to 280 KDa and expressed maximally in the neurons, oligodendrocytes, adrenal medulla as well as leukocytes. Although this protein is extremely conservative in mammals, but it has shown 99.4% homology with creatures like rats and dogs.

In a recent study, it has been reported that neurofibromin has a dimeric structure formed with the aid of C-terminal domain and tubulin-binding domain [3].

Neurofibromin consists of five major domains; CSRD, GRD, TBD, SEC PH and CTD [2]. These domains collectively contribute towards the regulation of the RAS-protein.

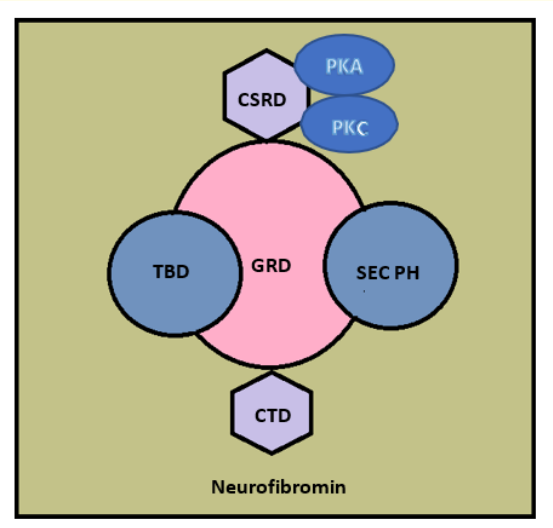

Figure 1: *Pictorial representation of the Neurofibromin domains. CSRD called as the cysteine-rich domain consists of 543-909 residues and is primarily phosphorylated by the PKA and PKC (Protein kinase A and C) that aids in the phosphorylates the GRD and RAS and also strengthens the activity of the actin protein. GRD known as the GAP related domain consisting of 1198-1530 residues that aids the hydrolyzation of the RAS protein thus making it inactive. Sec PH is a bi-domain protein with one part associated with the amino terminus and the other part resembles like the pleckstrin homology and is known to be associated with some other protein. TBD is known as the tubulin binding domain that shows an inhibitory effect on the RAS-GAP activity with aid of another protein colchicine.*

The GRD or Gap related domain consists of the GAP proteins that negatively regulate the activity of the RAS protein oncogene. But the question arises that why the GAP only regulate the RAS protein and not the other category of proteins? What makes them so specific?

Tubulin-binding domain is formed of 1095-1197 amino acids that creates a profound impact with the RAS-GAP activity by interaction with the microtubules.

RAS is a membrane-oriented protein that has affinity for the guanine-exchange factors that are activated in response to the signals generated by the Tyrosine-receptor kinases. Upon activation, the Guanine exchange factor converts the RAS (inactive) to RAS (active) that leads to the activation of the several downstream pathways related to proliferation, differentiation as well as motility. But the RAS is negatively regulated by another protein called as GAP which resides in the GRD domain of the neurofibromin [3]. The guanyl nucleotide exchange factors named RAS-GRPs are known to induce the Ras protein in the catalytic region consisting of the Ras exchange motif as well as CDC25 domain. The Ras GRPs are activated by the DAG proteins through a membrane-oriented mechanism. This RAS-GRP has proved to be a prominent viable drug target against various diseases.

Interaction between the RAS and PI3K proteins

The PI3K family of proteins are known to cooperate the RAS in cell survival and proliferation. These two proteins are interacted in an extensive manner and the RAS efficiently interacts with all three isoforms of the PI3Ks. There are three classes of PI3Ks; Class I plays a

major role in cancers and its subunits p110γ and p110δ are involved in the leukocytes. Class II is known to have sequence homology with the other two classes of PI3K and there is less information on their expression pattern. Class III consist of only one protein i.e., VPS34 and its physiological significance is yet to be determined. We will focus only the Class I PI3K and its interaction with the RAS.

PI3K consists of the p110γ subunit that is activated by the RAS by 20-fold which leads to the conformational change into the substrate binding site or by the specific changes in the plasma membrane. This is how the PI3K activated by the RAS proteins and that is also essential for the activation of the Rac, a Rho-protein that is utilised by the Ras for the oncogenic transformation [4].

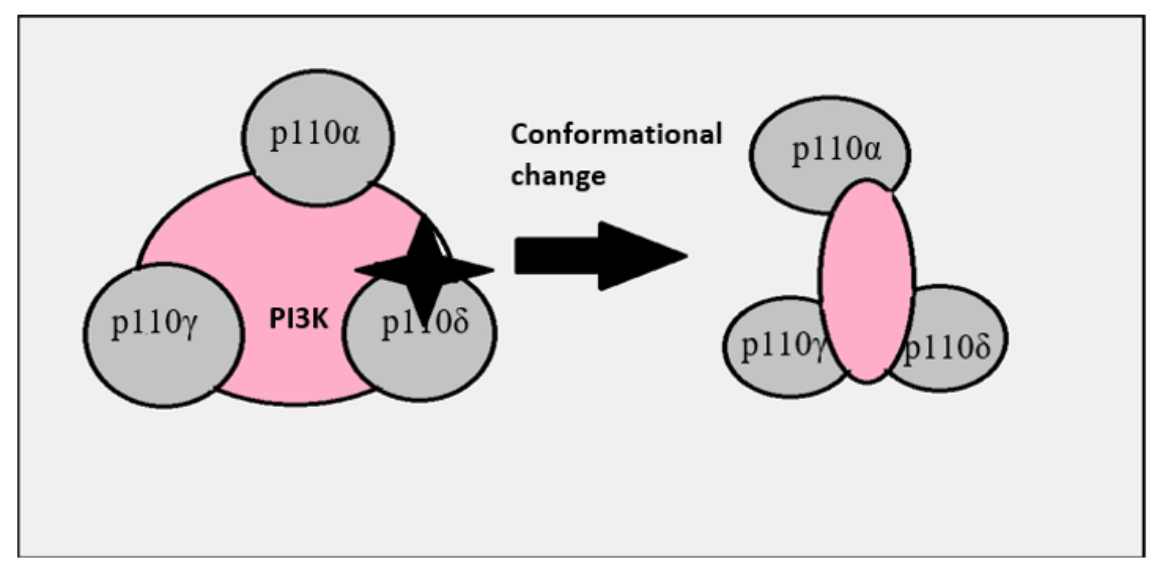

Figure 2: *Interaction of PI3K subunits with the Ras protein. The multidomain structured PI3K molecule has three subunits p110α, p110γ and p110δ. The Ras protein encounters the p110δ subunit that induces a 20-fold conformational change into the protein (right side). This conformational change is much needed to activate another critical protein "Rac" that is often used by Ras for being oncogenic in nature.*

Interestingly, neurofibromin de-regulates the RAS protein that make a huge impact on the interaction with the PI3K molecules. When the RAS protein is negatively regulated by the parent protein Neurofibromin suppresses the mTOR pathway and ultimately leads to the neurofibromatosis. The mTOR attenuates the activity of the RAS family of the proteins through the L--lysophosphatidic acid (LPA) as well as insulin [5].

Impact of the CRISPR modified neurofibromin on the PI3K signalling pathway

CRISPR Cas 9 technology is a game-changer in the field of the molecular biology. It is crucial to regulate the p110α subunit that directly interacts with the Ras protein in order to achieve better cell proliferation and controlled functioning in the PI3K pathway [4]. CRISPR engineered Neurofibromin studded with the Cas 9 protein will specifically inhibit the activity of the p110α with the p85 subunit that eventually strengthens the Ras protein with the aid of the phosphoproteins in the periphery. The Cas 9 protein will block the binding of the phosphoproteins.

A study reported that the activity of the phosphoprotein gets weakened upon the association with the p85 SH2 domain [6,10]. In this study, we expressed the Neurofibromin GRD and p120 GAP differently in the cells primarily obtained from the mutant mice. Here, the Cas 9 induced Neurofibromin GRD facilitated the normal growth in the mutant cells whereas the p120 GAP failed to do so. This may indicate the specific functioning of the CRISPR-engineered neurofibromin [7,8].

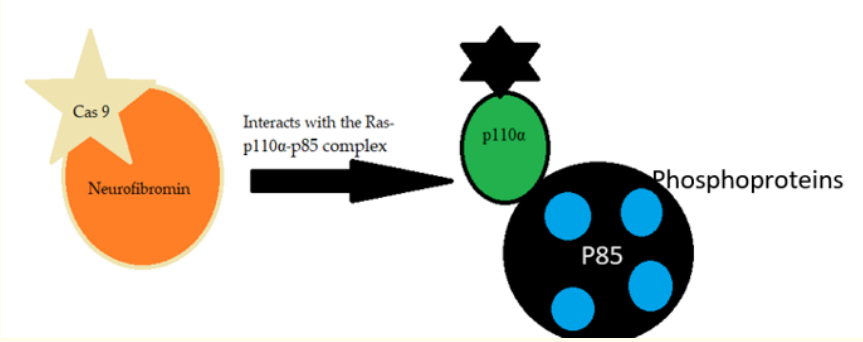

Figure 3: *Pictorial representation of the Neurofibromin-Cas9 molecule with the p110α-p85 complex encoded with the phosphoproteins. The Cas 9 molecule specifically target the phosphoproteins to block the association with the SH2-p85. The reduced affinity will ultimately weaken the interaction with the Ras proteins.*

Conclusion

Neurofibromin is quite known for its ability to perform negative regulation on the RAS molecules that play a crucial role in the functioning of the PI3K pathway. Neurofibromin is a multi-domain structured molecule that is known specific affinity towards the p110α subunit of the Ras protein. This p110α extensively interacts with Ras protein in the PI3K pathway.

This creates the need to specifically target this driver protein in order to regulate the functioning of Ras molecule. CRISPR Cas 9 technology can be utilized for the inducing the Cas 9 molecule into Neurofibromin plasmids that can specifically target the p110α that will block the interaction of the phosphoproteins with the SH2 of the p85 molecule [9]. While much research has already been performed on the interaction between the neurofibromin and the RAS but there is still need to understand the extensive molecular interactions taking place between the Neurofibromin and its specific target towards the phosphoproteins associated with the p85 molecule. The miraculous ability of the neurofibromin to show the targeted effect on the tumors is something that opens a wide variety of potential research opportunities.

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