



NEUROFIBROMATOSIS: A CAUSE OF CONCERN

Ramya Vishwanathan*¹, Vinod Kumar Gupta²

SRM Institute of Science and Technology, Kattankalatur, India,

²Rapture Biotech International Pvt. Ltd., Noida, India

*Corresponding author: svs1970@gmail.com

Received: 03-12-2021; Accepted: 12-03-2022; Published: 30-04-2022

© Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License <https://doi.org/10.55218/JASR.202213303>

ABSTRACT

Neurofibromatosis (NF) is a rare genetic disease which causes tumors to grow on the nerve tissues. These being non-cancerous and less life threatening, patients affected with NF can lead a normal life if the cosmetic issues are ignored. Although there are treatments available like surgeries or use of FDA approved drugs that can control the progression and symptoms in people affected with this disorder, researchers are still trying to find a potent solution that can permanently curb this disease. Novel technologies such as stem cell technology and CRISPR genome editing are being studied that could act as a cheaper, reliable, noncomplex and less time-consuming solution to this rare condition. In the current scenario, COVID-19 affected NF patients have neither shown progression of the NF condition or any severe side effects after being administered with vaccines. This review gives a gist of the drugs which are currently in use for treatment of this disease and various signaling pathways involved in the activation of Neurofibromatosis that could be inhibited using computational methods in the near future so as to obtain a solution which can be employed in the early stages itself.

Keywords: Neurofibromatosis, Neurofibromin, IVC therapy, Next-generation sequencing, Oncolytic measles virus therapy, Cabozantinib.

1. INTRODUCTION

Neurofibromatosis is a genetic disorder which is characterized by specific tumor growths on the tissues. These tumors originate from the supporting cells of the nervous system and can occur either because of family history or due to mutations of certain oncogenes during the early developmental stages. Neurofibromatosis being an autosomal dominant disorder, the chances of the offspring getting affected is around 50% with mild symptoms. Neurofibromatosis is a rare condition where approximately 1:3,000 children are affected with Neurofibromatosis 1 (NF1), 1:25,000 people with Neurofibromatosis 2 (NF2) and 1:40,000 people with Schwannomatosis. According to the recent annual reports, the mean mortality is around 0.92 per 1 million population [1].

As of now, there aren't any records showing increased risk of benign or malignant tumor growth and pain in NF patients affected with COVID 19. Since, COVID-19 vaccines are mRNA-based, they neither interact with the NF proteins (merlin, neurofibromin) nor modify the DNA in any way, making them safe for NF patients.

However, it should be noted that NF patients with lung impairment such as large plexiform neurofibromas in the chest cavity, chronic lung disease or severe scoliosis are very much prone to COVID-19.

There are currently three known types of this disorder namely, Neurofibromatosis 1 (NF1), Neurofibromatosis 2 (NF2) and Schwannomatosis.

2. CAUSES OF NEUROFIBROMATOSIS

Neurofibromatosis 1 (NF1), also known as Recklinghausen disease (named after the person who discovered it) is caused by a mutation that occurs on the Neurofibromatosis 1 (NF1) gene which codes for a cytoplasmic protein called Neurofibromin (320kDa) located on chromosome 17 which is a Ras-specific GTPase activating protein (RasGAP) [2]. Neurofibromin normally functions to down-regulate the p21 Ras oncoprotein. Lack of this neurofibromin leads to uncontrolled cell proliferation causing certain light brown coloured skin patches (café au lait spots) or soft bumps on the skin/peripheral nerves. It has also been recorded that Neurofibromatosis 1 (NF1) disorder is associated with RASopathies or RAS (Rat

Sarcoma Virus)/Mitogen-Activated Protein Kinase (MAPK) syndromes which are a group of phenotypically overlapping syndromes caused by germline mutations that encode components of the RAS/MAPK signalling pathway [3]. Recent discoveries show that novel gene variants like RIT1, RRAS, RASA2, A2ML1, SOS2 and LZTR1 are linked with RASopathies which are responsible in the progression of this condition [3]. It has also been documented using high resolution array-CGH that segmental duplications along the neurofibromatosis type 1 (NF1) gene locus on 17q11 mediate most gene deletions in NF1 patients [4]. Additionally, *in silico* studies show that out of the 16 non-synonymous SNPs (nsSNPs) in the RAS-GAP domain of neurofibromin, the K1444N (K1423N) mutation is the most pathogenic [5]. The recently generated human-derived neurofibroma model to study neurofibroma pathogenesis demonstrated that only NF1-null solute carriers (SLCs) were able to form neurofibromas with high levels of SOX10 expression, thereby showing the involvement of SOX10 in the origination of neurofibromas. Ablating NF1 in the Hoxb7 and Prss56 that serve as lineage markers to trace the developmental origin of neurofibroma neoplastic cells can recapitulate both human cutaneous and plexiform neurofibroma [6].

Another type of Neurofibromatosis is Neurofibromatosis 2 (NF2, acoustic neurofibromatosis or central neurofibromatosis) which is comparatively rare than Neurofibromatosis 1 (NF1) disorder. This is caused by mutation of the Neurofibromatosis 2 (NF2) tumor suppressor gene located on chromosome 22 that encodes for a cytoplasmic protein named Merlin that belongs to the ezrin-radixin-moesin (ERM) subgroup of the protein 4.1 superfamily, which links cell surface glycoproteins to the actin cytoskeleton. Merlin has a role in regulating the activity of multiple growth factors and so lack of this protein's functioning is responsible for tumor formation [7]. Since the tumor growths can be seen on the Schwann cells, this condition is also known by other names such as bilateral acoustic neurofibromatosis, vestibular schwannoma neurofibromatosis and central neurofibromatosis.

Schwannomatosis is a condition that occurs because of certain mutations on two genes namely SMARCB1/INI1 and LZTR1 located on chromosome 22. These two genes have a crucial role in tumor suppression and so their lack of functioning leads to tumor formation.

Generally, Hoxb7- and Prss56-expressing boundary cap cells/SCPs originate from a subpopulation of migrating NCSCs and represent the neurofibroma lineage of origin [6].

3. SYMPTOMS OF NEUROFIBROMATOSIS

Neurofibromin is responsible for regulating adenylyl cyclase activity by binding to microtubules that has a major role in cognition. Mouse models with neuron-specific NF1 loss have shown to cause these cognitive abnormalities. This NF1 loss leads to abnormal development of the cerebral cortex and astrogliosis along with an increased GABA-mediated inhibition and reduced hippocampal dopamine levels. This gives us an idea why cognitive impairment is also seen in Neurofibromatosis affected patients [8]. Apart from these, some patients may develop learning and behavioural disabilities along with scoliosis. This common disorder usually starts appearing in childhood and further may lead to complications like issues in pregnancy and high blood pressure. Moreover, about 12% of the patients affected with NF1 observe optic nerve gliomas in their initial stages of life that may further progress to visual loss or other neurologic symptoms [9]. Just like children are more prone to gliomas, adults are exposed to the formation of 2 major types namely, plexiform neurofibromas (pNFs) which are congenital and may progress into deadly malignant peripheral nerve sheath tumors (MPNSTs) or dermal/cutaneous neurofibromas (cNFs) which are not life threatening and generally appear at puberty that continue to increasingly grow in size and number on the skin [6].

Patients affected with Neurofibromatosis 2 (NF2) disorder start showing symptoms usually during their teen age. These include loss of hearing due to the pressure put by the tumors on the acoustic nerve, headaches, loss of balance, giddiness and vomiting sensations. Patients getting severely affected might also experience difficulty in swallowing, speech or vision along with vessel stenosis and sometimes development of meningiomas.

In case of Schwannomatosis, patients sometimes experience pain on the affected tissues (enlarged tumors) because of the pressure put on the tumor micro-environment. Additionally, patients might also experience numbness/weakness in the toes or fingers.

4. DIAGNOSIS OF NEUROFIBROMATOSIS

Neurofibromatosis can be diagnosed using the most common methods like Radiography, Genetic testing, Histology testing, X-rays, Computerized Tomography (CT) scans, Magnetic Resonance Imaging (MRI), tests of the biopsy samples and Ear, Nose, Throat (ENT) tests.

SMARCB1 and LZTR1 genes are quite close to Neurofibromatosis 2 (NF2) gene. Since these three genes show high rate of similarity in their mechanism and only differ in mutations, we can assume that Schwannomatosis

and Neurofibromatosis 2 (NF2) are similar conditions. Next-generation sequencing (NGS) technology can be used to differentiate mosaic Neurofibromatosis 2 (NF2) and Schwannomatosis [10].

Next-generation sequencing (NGS) can also be employed to target NF2, SMARCB1, LZTR1, SMARCE1 and SUFU tumor suppressor genes, using an amplicon-based approach [11].

5. TREATMENT OF NEUROFIBROMATOSIS

Neurofibromatosis disorder has no cure till date. Although, treatments are available that can reduce the proliferation rate and control the symptoms. These include removing the tumor growths by surgery, chemotherapy/radiotherapy in case of malignancy (not preferred for children) along with surgeries for scoliosis and cataract. It is advisable to regularly monitor this condition as the tumour may progress and turn malignant /cancerous. Although surgeries are an option for these conditions, they are not preferred much as they have multiple side effects. Researchers are constantly looking out for alternatives and success stories of some experiments have come into picture.

It has recently been discovered that a drug named Cabozantinib can be of great help for patients affected with plexiform neurofibromas. This drug acts as a tyrosine kinase inhibitor for the tumor cells and works on reducing the tumor proliferation and pain intensity in these patients with no severe symptoms. Despite this, more research has to be done on the drug for use in small age groups. Moreover, a Mitogen Activated Protein Kinase Kinase (MEK) inhibitor named Selumetinib has also given a positive response in some patients, particularly children. However, both Cabozantinib and Mitogen Activated Protein Kinase Kinase (MEK) inhibitors have not been successful in completely diminishing the existing tumors. Research is ongoing to use these two drugs to devise a combination therapy that can prove to be a possible solution [12].

Due to certain side effects recorded during chemotherapy of patients affected with optic glioma, drugs namely carboplatin and vincristine have also been administered. However, these drugs cause rashes and peripheral nerve damage in some people. The recently documented successful treatment of an optic glioma patient with intravenous vitamin C (IVC) that is followed by oral maintenance is coming into limelight. This IVC therapy could be used as an alternative one as it is non-toxic to normal cells, suppresses angiogenesis and inflammation and boosts the immune system as well. The ascorbic acid

involved in the treatment process plays a significant role in tumor cell proliferation and differentiation by shifting their epigenome and transcriptome. It is also observed that Vitamin D supplements act as useful agents in the differentiation therapy of human malignant gliomas [9].

The interplay between impaired NF1 protein (neurofibromin) inhibition of MEK/ERK-mediated SCP growth and mitogenic signals from non-neoplastic stromal cells has facilitated the identification of novel treatments for plexiform neurofibromas. Additionally, loss of neurofibromin in NF1, increases the expression of proteins in astrocytes. NF1-deficient astrocytes exhibit hyperactivation of rapamycin (mTOR) pathway, which could be inhibited by blocking K-RAS or phosphatidylinositol 3-kinase activation. This inhibition therapy can help restore the normal proliferative rates [13].

Malignant peripheral nerve sheath tumors (MPNST) cell lines highly express CD46 receptors for the measles viral entry. Local administration of human sodium iodide symporter (MV-NIS) into MPNST-derived tumors results in significant regression of tumor and improved survival. This gives us an idea that, oncolytic measles virus therapy (OMVT) for MPNST patients could be repurposed for NFI patients [14].

Moreover, NF1 patient-derived iPSC retinal ganglion cells (RGCs) can not only serve as a drug screening platform where genetically-inherited glaucoma, upon treatment with neuroprotective factors BDNF or PEDF exhibit a significant reduction in caspase-3 activation, but also could be used to replace degenerated retinal cells through autologous cell therapy for improvements in visual function. On the other hand, high-throughput screening assays have also been developed for iPSC-derived retinal pigment epithelium (RPE) that can be employed in the identification of therapeutic drugs for RPE-associated degenerative diseases [8].

Recent advances in Crispr/Cas9 gene editing approach show focal adhesion kinase (FAK) inhibitor GSK2256098 to be used for reducing the colony size in malignant meningioma cell line IOMM-Lee [15]. Additionally, the same approach has been employed to develop a novel rat model characterized with NF1-related pain. The cytosolic regulatory protein collapsin response mediator protein 2 (CRMP2) regulates activity of the ion channels and also binds to the targeted C-terminus of neurofibromin in a tripartite complex, suggesting a possible mechanism underlying NF1 pain. Prevention of CRMP2 phosphorylation with (S)-lacosamide resulted in normalization of channel current densities, excitability, as well as of hyperalgesia following CRISPR/Cas9

truncation of neurofibromin. Hence, CRMP2 is known to be a key target for treatment of NF1 pain [16].

Recent developments in iPSC reprogramming have the ability to generate human microglia-like cells (microglia which are non-neoplastic cells have a significant role in murine optic glioma tumors that have an immunogenic role delay tumorigenesis and reduce tumor proliferation). Further studies using *in vitro* co-cultures containing NF1-deficient human neuroglial progenitor cells in combination with NF1 patient-derived iPSC-microglia may reveal new targets for future stroma-directed low-grade glioma treatments. 3-dimensional (3D) organoid systems can prove to be excellent models for studying NF1-associated nerve pathology [8].

Anti-vascular endothelial growth factor (VEGF) antibody bevacizumab has shown efficacy for the treatment of neurofibromatosis type 2 (NF2). VEGFRs peptide vaccine uses cytotoxic T lymphocytes (CTLs) that have the ability to kill tumor cells and reduce the expression of VEGFRs in schwannomas with no side effects [17]. Moreover, it is reported that loss of NF2 gene leads to the induction of mitogenic signalling mediated by receptor tyrosine kinases (RTKs), MAP kinase, AKT, or Hippo pathways. Here, Her2, Her3, PDGFR β , Axl, Tie2 and YAP represent potentially valuable therapeutic targets [18].

Advances in the field of stem cell technology have shown the potential of stem cells in the treatment of neuropathic pain (NP). More research can lead to development of novel strategies employing stem cells for treatment of NF pain [19].

6. CONCLUSION

Neurofibromatosis is a rare autosomal dominant disorder that causes tumors to grow on the skin or nerve tissues. This can either be genetic or due to certain mutations on the genes. Since there aren't many *in vitro/in vivo* models available for studying the neurofibroma development and pathogenesis, novel mice models have been developed that could be useful for drug identification and screening. It is well understood that this condition cannot be cured, rather can only be treated for controlling the progression and symptoms. The available treatments have been discussed in this paper, some of which include surgeries, chemotherapies, CRISPR genome editing or using drugs like Cabozantinib and Selumetinib. A detailed study of the recently developed novel diseased models can give us an insight on the potent therapeutic peptides that can be used against the suitable discovered targets. Lastly, iPSC technology is emerging as a powerful field for studying

the neuro-developmental abnormalities in NF1, as this approach is capable of targeting patient-specific mutations and differentiating them into virtually any cell type that can help in understanding the NF pathogenesis.

7. ACKNOWLEDGEMENT

I would like to appreciate Rapture Biotech, India who even in this tough pandemic time gave me virtual guidance at each and every step to write this review article.

Conflicts of interest

The author declares that there is no conflict of interest.

8. REFERENCES

1. Masocco M, Kodra Y, Vichi M, Conti S, Kanieff M, Pace M, et al. *Orphanet journal of rare diseases*, 2011; **6(1)**:1-10.
2. Bonneau F, Lenherr ED, Pena V, Hart DJ, Scheffzek K. *Protein expression and purification*, 2009; **65(1)**:30-37.
3. Aoki Y, Niihori T, Inoue SI, Matsubara Y. *Journal of human genetics*, 2016; **61(1)**:33-39.
4. Mantripragada KK, Thuresson AC, Piotrowski A, Díaz de Ståhl T, Menzel U, Grigelionis G, et al. *Journal of medical genetics*, 2006; **43(1)**:28-38.
5. Agrahari AK, Muskan M, George Priya Doss C, Siva R, Zayed H. *Metabolic Brain Disease*, 2018; **33(5)**:1443-1457.
6. Mo J, Anastasaki C, Chen Z, Shipman T, Papke J, Yin K, et al. *The Journal of clinical investigation*, 2021; **131(1)**.
7. Golovnina K, Blinov A, Akhmametyeva EM, Omelyanchuk LV, Chang LS. *BMC evolutionary biology*, 2005; **5(1)**:1-18.
8. Wegscheid ML, Anastasaki C, Gutmann DH. *Experimental neurology*, 2018; **299**:270-280.
9. Mikirova N, Hunnunghake R, Scimeca RC, Chinshaw C, Ali F, Brannon C, et al. *The American Journal of Case Reports*, 2016; **17**:774.
10. Halliday D, Parry A, Evans DG. *Current opinion in oncology*, 2019; **31(6)**:562-567.
11. Louvrier C, Pasmant E, Briand Suleau A, Cohen J, Nitschké P, Nectoux J, et al. *Neuro-oncology*, 2018; **20(7)**:917-929.
12. Study demonstrates efficacy of new treatment for neurofibromatosis type 1-related tumors, www.sciencedaily.com/releases/2021/01/210114130119.htm, accessed on 22 November 2021.

13. Dasgupta B, Yi Y, Chen DY, Weber JD, Gutmann DH. *Cancer research*, 2005; **65(7)**:2755-2760.
14. Deyle DR, Escobar DZ, Peng KW, Babovic-Vuksanovic D. *Gene*, 2015; **565(1)**:140-145.
15. Waldt N, Kessler C, Fala P, John P, Kirches E, Angenstein F, et al. *Journal of Neuroscience Methods*, 2021; **356**:109141.
16. Moutal A, Yang X, Li W, Gilbraith KB, Luo S, Cai S, et al. *Pain*, 2017; **158(12)**:2301-2319.
17. Tamura R, Fujioka M, Morimoto Y, Ohara K, Kosugi K, Oishi Y, et al. *Nature communications*, 2020; **11(1)**:1-1.
18. Boin A, Couvelard A, Couderc C, Brito I, Filipescu D, Kalamarides M, et al. *Neurooncology*, 2014; **16(9)**:1196-1209.
19. Liu M, Li K, Wang Y, Zhao G, Jiang J. *Stem Cells International*, 2020:1-13.