Li-Fraumeni Syndrome: The Identification, Challenges and Future Prospects

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Li-Fraumeni syndrome (LFS) is a hereditary cancer predisposition syndrome caused by various mutations in the p53 gene, a tumor suppressor gene that is vital to prevent the transformation of healthy cells to cancer cells. Mutations in the gene have detrimental effects in terms of the gene's ability to suppress tumors, greatly increasing the chance of developing cancer. It is a rare syndrome affecting only about 0.025% of families worldwide and can result in various cancers. It often remains undiagnosed due to its rarity, lack of knowledge even among physicians, and lack of symptoms before the cancers manifest. This article provides an overview of this syndrome, focusing on its pathophysiology at the level of the p53 gene. It describes the history of the Li-Fraumeni syndrome, the diagnostic methods, and current and emerging treatment modalities for the syndrome including possibly gene therapy. This knowledge will increase the awareness of this rare hereditary syndrome which can have devastating consequences for the affected individuals. This research paper is inspired by works from Evans et al ("Li-Fraumeni Syndrome - A Molecular and Clinical Review"), which was crucial to understanding the various types of mutations that cause Li-Fraumeni syndrome; Moulder et al ("The Roles of p53 in Mitochondrial Dynamics and Cancer Metabolism: The Pendulum between Survival and Death in Breast Cancer?"), which contributed to the understanding of the tumor suppressing role of the p53 gene; and "A New Therapeutic Basis for Treating Li-Fraumeni Syndrome Breast Tumors Expressing Mutated TP53" from Glazer et al, which was used to determine how the mutation of the p53 gene in Li-Fraumeni syndrome affected its ability to suppress tumors.

Keywords: Li-Fraumeni Syndrome, cancer predisposition syndrome, mutation, p53 gene, tumor suppressor, cancer, pathophysiology, gene therapy

Introduction to Li-Fraumeni Syndrome (LFS)

Li-Fraumeni syndrome was discovered by Dr. Frederick Li and Dr. Joseph Fraumeni in 1969[1]. They first reported four families with children diagnosed with rhabdomyosarcoma (RMS) and other soft-tissue sarcomas in infancy[2]. They found that those families had a strong familial history for malignancies such as breast cancer that affected close relatives at a young age[2]. This was later studied by Birch, et al, who classified it as a clinical syndrome and named it as Li-Fraumeni syndrome[2]. Further studies in 1990 confirmed that LFS was linked to the p53 gene[2].

LFS is most commonly caused by different germline mutations, with approximately 70% of them being substitutions, in the p53 gene, a tumor suppressing gene that codes for the p53 protein. However, there have also been LFS families recorded to have a mutation in the CHEK2 gene, which is also a tumor-suppressor gene[3,4]. Evidence, however, suggests that the CHEK2 gene is not definitively involved in LFS since these mutations were discovered to either be polymorphisms or on a duplicated exon[4]. The mutations in the p53 gene are naturally occurring and, for children who do not inherit the syndrome, the mutations most likely formed in the egg or sperm cell. It is a genetic disorder with an autosomal dominant inheritance, meaning that if any one parent has the gene for the mutation, then each child will develop Li-Fraumeni syndrome when either parent passes down the gene[5].

LFS is a rare syndrome, affecting an estimated 5 in 20,000 families worldwide[6]. More than 90% of them develop cancer at least once and female breast cancers make up 27-31% of all LFS cancers, making it the most common symptom for LFS[6,7]. Exon 4 and intron 3 are the most frequently mutated in the p53 gene for breast cancer patients[8]. Despite the commonality of a p53 gene mutation among individuals with LFS, not all have a mutation on the p53 gene[5].

There is a similar disorder called Li-Fraumeni-like Syndrome, or LFL, which is like Li-Fraumeni syndrome, but does not satisfy all criteria. LFL is also caused by p53 and/or CHEK2 gene mutations and shares most of the phenotypes of LFS, though those mutations are different from those of LFL. LFL's p53 mutations are mostly point mutations like LFS's.

Symptomatology, Clinical Diagnosis and Care

Although individuals with LFS have reported symptoms of fatigue, anxiety, depressed moods, pain, and stress, LFS does not have any proven symptoms of its own other than higher chances of cancers, and cancers in general are not necessarily a result of LFS[9]. Cancers, however, could frequently develop in patients, and oftentimes patients face multiple cancer diagnoses throughout their lives[5]. Conversely, some patients may not ever develop cancer throughout their lives[5]. The time between first and subsequent cancer diagnosis is random. In fact, sometimes individuals can be diagnosed with two or more cancers at the same time due to the numerous tumors that could possibly develop from poor suppression of tumors by the p53 gene[10].

LFS can only be definitively diagnosed by genetic tests. However, before any genetic tests are conducted, the Classic LFS criteria are used to aid with the clinical diagnosis of LFS, with the first being having a sarcoma before attaining 45 years of age[11]. Next, there must be a familial history (parents, siblings, children) of cancer of any form by the age of 45[11]. Finally, second-degree relatives and other first-degree relatives (including grandparents and grandchildren) must be diagnosed with any cancer by the age of 45[11].

The Chrompet criteria is a common and more recent set of criteria used to distinguish or diagnose LFS beyond the Classic LFS criteria[11]. In the Chrompet criteria, one of the three criteria must be met in order to confirm LFS diagnosis[11]. This includes, firstly, a tumor belonging to the LFS tumor spectrum by the age of 46 and at least first- or second-degree family members with an LFS-related tumor before the age of 56 with multiple tumors (except if the tumor was breast cancer if the individual being diagnosed had breast cancer); next, a person with multiple tumors (except breast tumors), two of which belong to the LFS tumor spectrum with the first one having occurred before the age of 46; and finally, a person diagnosed with adrenal cortical carcinoma/tumor in the choroid plexus (a membrane around the neck), regardless of family history[11].

If an individual does not meet either criteria for LFS, they may be diagnosed with LFL through either the Birch definition or Eeles definition of LFL[11]. The Birch definition states that all of three criteria must be met for LFL diagnosis, with the criteria being a proband with any childhood cancer or sarcoma, brain tumor, or adrenocortical carcinoma that was diagnosed by the age of 45; a first- or second-degree relative having a typical LFS cancer at any age, and a first- or second-degree relative having any cancer by the age of 60[11]. The Eeles definition has one criteria that must be met for LFL diagnosis, which is two first- or second-degree relatives having LFS-related cancers at any age[11]. It should also be noted that LFL, unlike LFS, does not require genetic testing to be diagnosed and is rather only a clinical diagnosis[11], and it is not specific to the p53 gene as only about 22% of families meeting either LFL criteria carry p53 gene mutations[11].

Individuals may be tested at birth if there is a known family history of LFS. However, without a family history, the condition will remain undiagnosed until cancers develop. For the genetic tests, a blood or saliva sample is obtained from the patient, then the DNA is isolated from the sample and both copies of the p53 genes are sequenced using DNA sequencing[11,12]. In this method, the p53 gene of the patient and the reference copy of a p53 gene are compared to identify any mutations and also to identify whether such mutations are associated with LFS[11,12].

If an individual test positive for LFS, the standard of care for monitoring them includes periodic cancer screening. This would include a recommended schedule for children under 18 for full physical examinations, complete blood count (CBC), abdominal and pelvic ultrasounds, and urinalysis every three to four months as well as brain and body MRIs every year[6]. These check for blood cancers and different tumors that may have developed as a result of LFS. For adults, the recommended screening schedule is as follows: monthly breast self-examination, general physical examinations every six months, yearly breast, brain, and whole-body MRIs along with abdominal and pelvic ultrasound, yearly full-body skin check, and upper endoscopy and colonoscopy every two or three years[6].

Patients who do develop cancers may be treated with various modalities including chemotherapy, which is the usage of drugs, such as alkylating agents that function by damaging cell DNA and antimetabolites that prevent cancer cells from making the genetic material necessary to create new cells, to kill the cancer cells[13]; radiation therapy, which uses high energy beams to kill the cancer cells; surgery, which is used to remove as much of the cancer as possible; and immunotherapy, which uses the body's immune system to fight the cancer[14].

Research Methodology

This research was mainly conducted as a literature review. The papers from Evans et al, Moulder et al, and Glazer et al contributed to the crux of the results, providing the data and information for the figures and the corresponding analyses[15–17]. The paper by Evans et al provided information regarding the list of mutations that have been discovered to occur in the p53 gene for LFS and similar syndromes, therefore enhancing the knowledge in such activity that occurs in the genetic level to cause LFS[15]. By explaining the function of the p53 gene, Moulder et al contributes to information about how the gene is supposed to function by detailing the pathway of the gene's function of suppressing tumors[16]. Finally, Glazer et al details the differences between the wild-type and LFS mutant p53 genes and how they function, hence aiding to explain the numerous effects of the mutations that cause LFS[17].

These papers were selected from Google Scholar as they provide valuable data and information regarding the mutations

that cause Li-Fraumeni syndrome, the role of the p53 gene in cancers, and determining the effects of the mutation of the p53 gene in Li-Fraumeni syndrome.

Results: Analysis of LFS on the Genetic Level

In the p53 gene, which is located on chromosome 17, there are many different loci where the mutations of LFS and LFL may occur. LFS can also be caused by different types of alterations in the same gene. For instance, a deletion, insertion, or point mutations may occur, though most of the known mutations that result in LFS are point mutations [15].

LFS and LFL account for the majority of the different types of p53 mutations that lead to cancer predisposition syndromes. Approximately 50% of these mutations lead to LFS, and approximately 20-30% lead to LFL [15]. The rest of the mutations lead to syndromes not conforming to either [15]. As previously stated, most of these mutations leading to LFS are point mutations (including both transitions and transversions), though there are also other types of mutations leading to LFS like insertions and deletions [15].

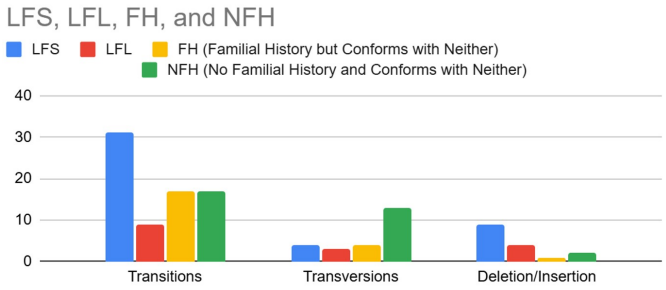

Fig. 1 Mutations of the p53 gene in LFS and associated syndromes [18]. FH means that the syndrome does not conform to LFS or LFL but there is a family history for cancer, and NFH means that there is no family history and it does not conform to LFS or LFL [15].

Based on a study of the p53 mutations that cause LFS and associated syndromes, shown in Figure 1, out of the 44 instances of mutations found in LFS, about 72% (31) were transition mutations in the p53 gene, which involve the change from a purine nucleotide base to another purine base or a pyrimidine base to another pyrimidine base [18]. However, for LFL patients, out of 16 instances, approximately 56% (9) were transitions, and for mutations that conform to neither LFS nor LFL, about 63% (34) of 54 instances were transition mutations, and this includes both patients with and without family history. Transversions, the change from a purine base to a pyrimidine base or a pyrimidine base to a purine base [18], only make up about 9.1% (4) of LFS mutations, 19% (3) of LFL mutations, and 31% (17) of mutations conforming to neither. Most of the transitions occur on exons 4-8 on the p53 gene. This means that a single point mutation may be crucial to determine whether an individual has LFS, LFL, or neither. Deletions and/or insertions make up approximately 20% (9) of LFS mutations, 25% (4) of LFL mutations, and only about 5.6% (3) of mutations conforming to neither.

The p53 gene makes the p53 protein, which is like other proteins in terms of structure as it is made up of a polypeptide chain in its primary structure and β-pleated sheet for the secondary structure, and it has a DNA-binding domain and a stabilizer, which, in this case, is a zinc atom [19]. When there is no mutation, the p53 gene regulates cell division and death in which it causes cell cycle arrest to damaged or unnecessary cells so that it could either repair the damaged cell or cause cell death (See Figure 2) [16]. This leads to only undamaged and necessary cells to sustain and carry out cell division. As a result, all the cells would be healthy ones that the body needs, and the damaged cells would not divide, therefore avoiding cancer.

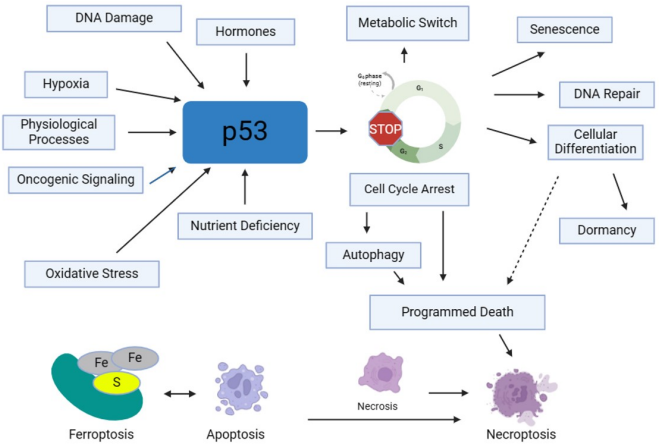

Fig. 2 The central role of p53 in cell survival and death [16]. Nutrient deficiency, hormones, DNA damage, oncogenic signaling, hypoxia, oxidative stress, and/or physiological processes all activate the p53 gene via cell cycle arrest to such cells. This causes senescence, a metabolic switch that involves differentiation and hence redirects specialized cell functions, DNA repair, a stop to cellular differentiation via programmed death, and programmed death itself. Programmed death may also result from autophagy. This proper functioning of the p53 gene allows the cell to enter cell cycle arrest and cell death when necessary and when corrections cannot be made while the useful cells remain in the body [16].

P53 plays a central role in cell death and survival by controlling whether the cell enters apoptosis or whether it should continue to live. It activates caspase 3, which is what regulates apoptosis of the cell [17]. As a result, cells that do not function properly or are unnecessary would experience apoptosis when caspase 3 is activated. This avoids cancer as cancer is caused by none other than the lack of or poor regulation of cell division and cell death: without caspase 3, cells would uncontrollably divide with a lack of cell death for the cells that otherwise will

and must die, leading to cancer (See Figure 3).

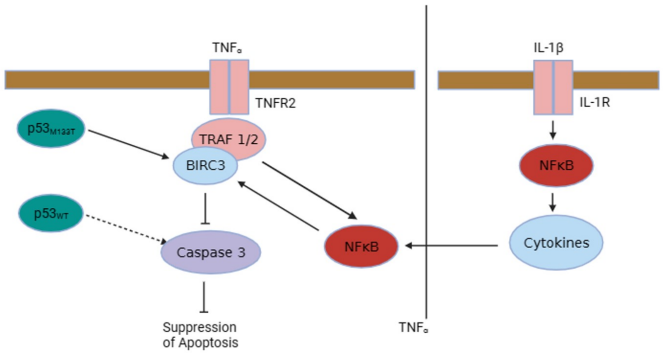

Fig. 3 p53 wild type and mutant genes regulation pathways[17]. A p53 gene with the M133 mutation would upregulate BIRC3 expression, which would associate with the TNFα receptor to TNFR2-associated proteins, TRAF 1 and TRAF 2, to inhibit the activation of caspase 3, hence suppressing apoptosis. TRAF 1 and 2 also upregulate the transcription factor NFκB, increasing BIRC3 expression. The stroma cells of LFS patients upregulate IL-1β, inducing NFκB and then leading to cytokine secretion that further continues NFκB expression and pro-survival signaling[17].

Conclusion

The p53 gene is crucial in the human body, and even a minor genetic mutation in the p53 gene can cause LFS, LFL, or other cancer predisposition syndromes. Such syndromes can be transmitted hereditarily, leading affected families to suffer with various cancers. The p53 gene is supposed to function in a way that suppresses the tumor: it activates cell cycle arrest for cells that are defective and attempt to repair the DNA or carry out apoptosis, hence preventing the propagation of the defective cells. The LFS and LFL mutations, however, hinder the ability of the p53 gene to carry out this regulatory function by inhibiting caspase 3, hence disabling the p53 gene's ability to carry out apoptosis whenever necessary. This leads to uncontrolled division and survival of the defective cells, causing cancer. Awareness of these syndromes is crucial to be able to combat such a rare and deleterious syndrome and enable early diagnosis and preventive and therapeutic strategies. Families with familial cancer history must be tested for any genetic mutations so that such syndromes can be identified.

Future Work

At the present time, the management of LFS is limited to dealing with the cancers that develop with the syndrome. However, as a genetic syndrome, LFS could be curable with gene therapy, a technique used to modify the person's genes. A gene therapy for LFS could involve administration of a functional copy of the p53 gene. In the case of LFS, it may be also possible to modify the p53 gene's mutation. Clinical studies recently have taken the approach of immunogene therapy, where immune-stimulating agents activate the body's defense mechanisms[20]. These agents are introduced to the cell via viral vectors, which is what modifies the genes.

The vaccine used in the trials was INGN-225, a dendritic cell-based p53 gene vaccine that delivers a p53-modified adenovirus to the body to carry out gene therapy, and it proved to provide an immune response in lung cancer patients with p53 gene mutations via differentiated effector T cells attacking and destroying the malignant, p53-overexpressed cells[21]. This therapy is still being developed and, despite the relative novelty of this technique, it could revolutionize the treatment of p53 mutations.

There could also be vaccinations introduced in order to prevent symptoms of p53 mutations, though they would not be able to prevent the mutation itself, since that would be inherited or occurring before birth. There already exists some cancer vaccinations, specifically therapeutic cancer vaccinations, that help prevent cancers by targeting the p53 gene, and this is known as immunotherapy as it mobilizes the immune system and helps it recognize, attack, and destroy cancer cells[20].

A recent invention is Novartis' Locametz, an imaging agent that helps easily identify where there are cancers[22]. This is an injection that introduces gallium-68 to the body, which can be found glowing in the PET scans wherever there are tumors[22]. Novartis also introduced Pluvicto as a radioligand therapeutic treatment method[22]. Here, the treatment combines a ligand, or a targeting compound, with a therapeutic radioisotope[22]. Pluvicto is administered into the bloodstream, after which it binds to target cells including prostate cancer cells that express prostate-specific membrane antigen, or PSMA[22]. This leads to energy emissions from the radioisotope damaging the target cells and nearby cells, hence avoiding their replication and possibly triggering cell death[22].

Artificial intelligence (AI) can and already does play a large role in treating p53 gene mutations and the cancers they result in. Scans, such as CT scans and PET scans, have already been developed to identify cancers in the body[23]. CT scans, or computer tomography scans, use multiple x-rays to create a detailed image of the internal body[23]. Like x-rays, they involve exposure to low-dose ionizing radiation[23]. PET scans, or positron-emission tomography scans, however, use radioactive substances called radiotracers to find abnormalities in the body's metabolism[23]. Novartis' Locametz, as explained previously, uses PET scans to identify the cancer cells[22].

AI can still be revolutionized with ways to cure cancer[24]. Despite drawbacks of possible overreliance of AI, it can increase accuracy of diagnoses and help for clinical decision-making as it can recognize patterns in large volumes of data, find re-

lationships between complex features in the data, and identify characteristics in data that cannot be perceived by the human brain[24]. In fact, currently, radiologists use AI to detect breast cancer after the FDA approved the first AI-based software to rapidly process images and help the radiologists to detect breast cancer in screening mammograms[25]. Such inventions and future possibilities lead to biomedical engineers being in high demand.

There is a new therapeutic approach that would selectively inhibit the tumors expressing the phenotype of the mutated p53 gene. Here, LPS-50 cells are treated with CP-31398 (Figure 4 - See Appendix)[26] or PRIMA-1 (Figure 5 - See Appendix)[27–29], which interrupt the mutated p53 gene's signaling and convert from mutated to wild type p53 conformation. This could result in an inhibitory effect on BIRC3 expression and a reduction in cell growth. Overall, this approach would practically force cells to go into apoptosis in tumors from patients with any p53 genetic mutation including LFS and LFL. Although CP-31398 and PRIMA-1 show promise theoretically, they are both in the early stages, with CP-31398 not having progressed to clinical trials and possibly being abandoned for more promising candidates and PRIMA-1 in the early clinical trials stage[27,30,31].

Monoclonal antibodies is another rising therapy, especially for leukemia from p53 gene mutations[6]. Trastuzumab, one of such monoclonal antibodies, has been previously used to cure breast and stomach cancer and is still in use for several malignancies[25]. In this case, Trastuzumab inhibits cell growth by binding to the extracellular domain of the human epidermal growth factor receptor type 2, and this leads to the patient's immune system targeting the cancer cells[25].

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Appendix

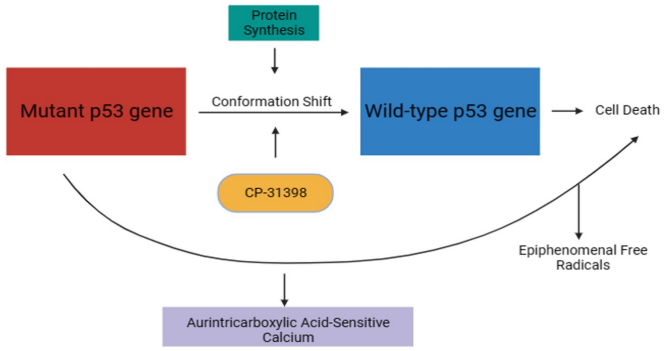

Fig. 4 Role of CP-31398 as a drug candidate to cure cancers from p53 gene mutations [26]. CP-31398 is a styrylquinazoline that stabilizes the p53 gene's active conformation in order to promote its activity in the gene. This restoration of the wild-type DNA-binding conformation of the mutant p53 allows for the suppression of the individual's tumors. There are two pathways for its CP-31398-induced cell death, including a p53-dependent one requiring protein synthesis and a p53-independent one where there is a release of aurintricarboxylic acid-sensitive calcium and a formation of epiphenomenal free radicals. Despite its liabilities, it was one of the candidates for drugs to cure p53-mutated cancers [26].

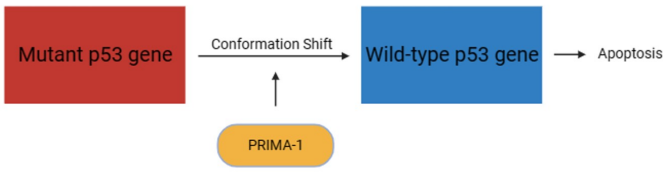

Fig. 5 Role of PRIMA-1 as a drug candidate to cure cancers from p53 gene mutations [27–29]. PRIMA-1, a small molecule therapy, converts into its methylated form, PRIMA-1. PRIMA-1 does a conformation shift to the mutant p53 protein by covalently bonding to the thiol groups and modifying them in the protein's central domain. This would reactivate the protein and allow it to carry out apoptosis when necessary, hence reinstating the function of the wild type p53 gene's protein. It is currently in Stage II clinical trials for a possible alternative to chemotherapy [27–29].