Literature review

Current and Novel Directions in Fanconi Anemia Treatment Methods - a Literature Review

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Keywords: Fanconi anemia, gene therapy, stem cell transplant, hereditary anemia, needs-led research

Abbreviations: FA - Fanconi anemia, AD - autosomal dominant, BMF - bone marrow failure, HNSCC - head and neck squamous cell carcinoma, HSCs - hematopoietic stem cells, HSCT - hematopoietic stem cell transplantation, HLA - human leukocyte antigens, RBCs - erythrocytes, iPSCs - induced pluripotent cells, EGFR - epidermal growth factor receptor, CRISPR - clustered regularly interspaced short palindromic repeats; Cas9 - CRISPR associated protein 9, ZFN - Zinc finger nucleases, TALEN - transcription activator-like effector nuclease.

Abstract

Fanconi anemia is a genetically-transmitted disease caused by biallelic inactivating mutations in more than 20 genes. While its main symptoms include severe anemia, other cytopenias and skin pigmentation impairments, the aspect which concerns both patients and caregivers is the connection between Fanconi anemia and the development of several malignancies, especially breast, skin and blood-related cancers, as well as squamous cell carcinomas. Current treatment methods rely on anemia correction through stem cell transplantation, however it has become more and more evident that modern and more efficient therapies need to be developed. This needs-led literature review provides an overview of the main highlights in Fanconi anemia current and emerging therapeutic directions in order to improve the patients' quality of life.

1. Introduction

Fanconi anemia (FA) is a genetic disorder characterized by anemia and other cytopenias, short stature, digital abnormalities, as well as café-au-lait spots and other pigmentation disorders.[1] Biallelic inactivation of genes in

the FA pathway result in malfunction of hematopoietic stem cells (HSCs) and bone marrow failure (BMF), classifying FA as an inherited Bone Marrow Failure Syndrome.[1,2] BMF develops in up to 86% of FA patients by age 6.6 and the median age at diagnosis is 7 years old.[3,4] FA is part of the rare diseases group, being identified in 1/300 000 live births, with a prevalence of 1-9 per million.[5]

FA predisposes to a number of malignancies such as head and neck squamous cell carcinoma (HNSCC), which can be found in up to 14% of FA patients that survive to the age of 40.[6] Breast cancer, skin malignancies, cervical cancer and leukemia are also associated with FA.[3] Children with FA have a risk of developing cancer 39 times larger than the one of

the general population, with up to 11% of patients developing cancer by the age of 18 and 20% of patients developing solid tumors by age 65.[6,7]

Signs and symptoms of FA include short stature (43% of patients), pigmentation disorders including café au lait macules (37% of cases), renal malfunctions (27% of patients), upper limb abnormalities (40% of cases), microcephaly (27%), as well as hypogonadism and gastrointestinal abnormalities.[1,4] Up to 25% of patients present no typical clinical features. Clinical suspicion is followed by cytogenetic or molecular tests, confirming the pathology. Differential diagnoses may include Diamond-Blackfan anemia, Shwachman-diamond syndrome and Evans Syndrome.[5]

Studies conducted regarding the genetics of FA showcased that the etiology of this rare disease lies in biallelic inactivation-resulting mutations on 21 genes found on the following chromosomes: 1 (1q31.3, 1p36.22), 2 (2p16.1), 3 (3p25.3), 6 (6p21.31), 7 (7q36.1), 9 (9q22.32, 9p13.3), 11 (11p14.3), 13 (13q13.1), 14 (14q21.1), 15 (15q26.1, 15q15.1), 16 (16q24.3, 16p12.2, 16p13.3, 16p13.12), 17 (17q23.2, 17q22, 17q21.31) and X (Xp22.2).[8,9] In almost all cases, it presents an autosomal recessive transmission, except for when it is associated with FANCB gene alterations on the X chromosome, or autosomal dominant alteration of the FANCR gene, which results in an AD transmission.[10,11] Proteins encoded in each of the FA genes play an important role in genome stabilization, while creating a specific molecular pathway proved to interfere with not only tumor-producing pathways, but also normal biological phenomena such as body response to oxidative stress and inflammation.[9] Studies have shown that FA proteins stimulate down-regulation processes of antioxidant protection genes, suppressing inflammasome activation and production of inflammation-implied cytokines.[12]

Nowadays, multiple therapeutic methods are being considered and the surveillance for cancer development is needed for all of them, as FA and other HSCT-requiring diseases still impose an economic burden on healthcare systems worldwide, while also physically and psychologically affecting the patients' community.

2. Materials and methods

Articles published between 2017 and 2023 were sourced on PubMed. Clinical trials, systematic reviews and meta-analyses studying cell, gene and pharmacological therapies were included. Treatments used at a large scale, as well as emerging therapies were studied. Inclusion criteria used were hematologic results of treatments, complications and side effects, symptom control and life expectancy of patients. Exclusion criteria used were phase 1 clinical trials, studies on animal models, therapies that are no longer in use.

Keywords used in the search were "Fanconi anemia", "treatment" and "therapy". The search formula used was "Fanconi anemia AND (treatment OR therapy)". Search results were analyzed by all authors, and statistically relevant ($p<0.05$) data was collected. Only articles written or translated in English were included. SANRA guidelines were followed and bias risk was not assessed.

2424 articles were found, out of which 134 met the year of publication and article type criteria. 24 articles were selected according to the inclusion criteria. For the Introduction, 3 comprehensive reviews were separately included, regardless of the year of publication.

3. Current treatment methods

3.1. Hematopoietic stem cell transplantation (HSCT)

Fanconi anemia is considered an inherited bone marrow failure syndrome due to hematopoiesis impairment. Platelets count over 30 x 10^9 pl/L is considered to be the bottom limit and it can be tolerated for years. However, multiple complications may arise and the hematopoietic stem cell transplantation (HSCT) is considered the optimal choice for inducing normal hematopoiesis. Both the hematopoietic manifestations and the risk of leukemic transformation are corrected with stem cell transplantation. Since the procedure itself is complicated, the prophylaxis of infections should represent a priority and the procedure should be performed only if needed, in cases such as bleeding, transfusion dependency or infectious complications evolving. The risk for cancer development should as well be monitored.[13]

In most of the cases of FA, transfusion of packed erythrocytes (RBCs) and platelets is performed. For patients with leukopenia, treatment with granulocyte-colony-stimulating factor (G-CSF) generates an optimal response - however, this response is limited in patients having an absolute neutrophil count less than 200/mL.[14]

Best outcomes have been reported in the cases of people undergoing HLA - matching family or unrelated transplants. For patients without a perfect donor, a partially mismatched donor can be used.

Factors such as the age at transplant and the components of the conditioning regimens influence the outcomes. According to a clinical trial conducted by Bierings et al.[15], out of 199 patients transplanted 18 developed secondary malignancies - 8 solid tumors and one *de novo* leukemia. Rio et al. suggest that better chances of survival were recorded for patients that had a high compatibility with their donors, but also for patients that were administered fludarabine in the conditioning regimen and for patients where T-cell depletion strategies were included in donor grafts.[16] According to E. Carreras et al., current conditioning regimens generally contain *fludarabine* (cumulative dose 150 mg/m^2), in combination with reduced doses of *cyclophosphamide* (cumulative dose 50 mg/m^2) and *total body irradiation* (100–300 cGy) in cases of unrelated donors.[17]

The most concerning outcome is the development of secondary malignancies, especially squamous cell carcinoma in the aero-digestive and ano-genital regions especially in the third and fourth decade of life, which is why regular ear, nose, and throat specialist checks for early signs of malignant transformation are needed. In 2003, IFAR reported that cancers were frequently affecting the oral cavity (68%), followed by larynx and oropharynx, each with 11%.[18]

Apart from malignancies, non-hematological problems such as infections post-transplantation, graft-versus-host disease, endocrine dysfunction (hypothyroidism, growth failure, early menopause and infertility) or impaired heart, lung or kidney function were recorded. For FA patients with head and neck squamous cell carcinoma (HNSCC), the treatment should be adapted because chemotherapy induced toxicities are definitely more frequent and severe in these patients and healthy tissues are affected, including the bone marrow transplanted.[19] Surgery seems to be generally well-tolerated - according to a literature review conducted by Beckam et al.[18], 8 out of 9 revised clinical cases underwent a surgery, and 2 of them developed complications: one had postoperative tachycardia and respiratory distress which were related to other sources of comorbidities and another one with pancytopenia at the time of surgery had wound healing and infectious complications.

However, donor stem cell material for hematopoietic transplantation is a limited resource and other sources of allogeneic or patient-specific hematopoietic stem cells have been considered, such as umbilical cord blood and induced pluripotent cells.[20] The main advantage of the umbilical cord blood would be the fact that the donor's health risk is minimal and it only requires the donor and the recipient to be partially matched.

3.2. Pharmacological approach

Androgen therapy is the most common pharmacological therapy which normalizes the blood in up to 50-70% of FA patients. Common androgen products, such as oxymetholone, stimulate the bone marrow to produce more RBCs and platelets. For some patients, they also stimulate the production of neutrophils. Unfortunately, androgen therapy is not a long-term solution and it is eventually replaced with HSCT.[20]

4. Emerging therapies

There is a high number of therapeutic guidelines for the treatment of FA-related malignancies in current usage and in testing phase, such as cetuximab and other EGFR inhibitors in FA-HNSCC or poly ADP-ribose polymerase inhibitors (PARPi) in pancreatic or breast cancer and others.[21,22,23] However, only a small part of these actually target the disease at its origins. Current research mostly focuses on the development of gene therapies, genetically engineered HSCs, as well as the repurposing of existing drugs.

4.1. Treatments based on gene transfer

Multiple clinical trials have demonstrated the efficacy of viral vectors for the *in vivo* incorporation of FA genes into the patients' genome. Currently, the most used are LVVs - Lentiviral vectors, which are retroviruses, as they present higher safety and efficiency levels rather than adenoviruses.[24] LVVs have also shown great success in gene therapy targeting other diseases, such as beta-thalassemia[25] or sickle cell disease, which provides researchers with hope for this method to be effective in the treatment of FA on a larger scale.[24]

4.2. Treatments targeting genome editing

Due to the fact that FA also interferes with DNA repair, in vivo genome editing would be highly impractical. However, genome editing techniques such as CRISPR/Cas9, ZFN (Zinc Finger Nucleases) and TALEN can be used effectively in modifying HSCs in preparation for transplantation procedures. A recent study revealed that genomically edited HSCs have the potential to restore FA genes function, with no evidence of unwanted mutagenesis.[24,26]

4.3. Pharmacological repurposing

The repurposing of several drugs, which provides them to be used in the treatment of different diseases than they were originally developed for is no news to the scientific community. A recently conducted study proved that metformin, a drug usually used in the treatment of type 2 diabetes mellitus, is effective in the treatment of cytopenias caused by FA, while also targeting the FA-induced insulin resistance. It has also been shown that metformin treatment reduces the risk of solid malignancies development. However, further research needs to be conducted in this direction.[27]

5. Discussions and conclusions

Even though treatments currently performed at a large scale are efficient in terms of symptom remission in FA patients, they only represent a limited resource and are associated with secondary malignancies, post-operative infections, as well as graft-versus host disease. Non-surgical treatment only offers temporary symptom relief. Emerging therapies targeting the disease at its origins mainly focus on gene transfer and genome editing, aiming to fix the genetic anomalies causing the disease. Out of those, LVVs and CRISPR/CAS9, ZFN and TALEN have proven to be strong candidates in developing future curative therapies for FA. More research is needed, including late stage clinical trials, before these new therapies can prove to cure FA, however these novel therapeutic methods pose a great potential of improving the patients' quality of life and overall health.

Author contributions

These authors contributed equally to this work. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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