IL-25 Gene Delivery; Immunotherapeutic Approach for Cutaneous Squamous Cell Carcinoma

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Abstract

Squamous Cell Carcinoma (SCC) is a type of cancer, in which, mainly, skin, mouth and esophagus are pronto being affected. As a specified type of this carcinoma, Cutaneous Squamous Cell Carcinoma (CSCC) is regarded as the second most common non-melanoma skin cancer. About 250,000 cases of CSCC, are diagnosed annually, meanwhile, statistics depict a dramatic upsurge that culminated to 5 million in 2011. Classical risk factors of CSCC comprising age, ethnicity, skin phototype, Ultraviolet (UV) exposure, and immunocompromised. In such a complex disease, what turn out to be controversial, are uncompleted recovery and remained side-effects by common and previous therapeutic methods. Resistance to the apoptosis process and oscillations in cytokine profile (TNF-α, IL-24, NF-κB) occurred at the tumor microenvironment, which is emerging hallmarks in the immunopathogenesis of CSCC. T cell immunity interferes with programmed death-1 (PD-1) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) in tumor progression. Targeting cytokine profile and immune cascades seem to be an appropriate therapeutic and diagnostic modality for CSCC. These have been led to encourage basic clinical scientists to new horizons of clinical applications with immunological perspectives such as special immune biomarkers, stem cells, and vectors, which sound to be easily-accessible with diminished toxic effects in clinical trials. Numerous pieces of evidence propose that Interleukin-25 (IL-25) has important roles in immune system regulation and cell apoptosis.

Keywords: IL-25; Cutaneous squamous cell carcinoma; Apoptosis; Clinical applications; Mesenchymal stem cell; Immunotherapy.

Likewise, it has been proved that Adeno-Associated Viruses (AAVs) as vector and Mesenchymal Stem Cells (MSCs) that work in a coordinated manner to handle anti-tumoral effects. Overall, here we suggest a hypothetical gene and cell-based immunotherapy approach for the pro-apoptotic effect of IL-25 gene delivery via AAV by MSCs in a syngeneic dimethylbenz[a]anthracene/12-o-tetradecanoylphorbol-13-acetate (DMBA/TPA) mouse model of CSCC. Studies have shed light on the importance of convincing implication, which could be a reliance for oncologists to tackle their obstacles in treatment and for patients to qualify their lifestyle.

Introduction

Squamous Cell Carcinoma (SCC) is a type of carcinoma that can be occurred in various organs of the body such as the skin, lips, mouth, esophagus, bladder, prostate, lung, vagina, and cervix. This accounts for a malignant tumor of squamous epithelium, in which, its invasive types can metastasize other organs [1]. Correspondingly, SCC can affect the skin tissue and create Cutaneous Squamous Cell Carcinoma (CSCC). CSCC is the second common Non-Melanoma Skin Carcinoma (NMSC) that includes 20% of all these types of skin cancers with harboring high mutation burdens and high mortality rate in those developing nodal metastasis [2]. About 250,000 cases of CSCC are diagnosed annually and the treated population can exist in. But, a constantly growing rate of progression should be taken into account, too. Its diagnosis trend, depending on frequent pathological biospies and clinical tests, is complicated and always accompanied by several erroneous and inaccessible laboratories based diagnosis due to the similarity of a wide range of lesions related to this kind of cancer. In such complex disease, what turn out to be more controversial, are uncompleted recovery and remained side-effects by common and previous therapeutic methods as chemo or radiotherapy, bringing about problematic challenges and concerns [3-4]. CSCC is seen in unhealthy skin with chronic lesions such as lung-standing ulcers, sinus tracts, burns, and osteomyelitis. Also, it is observed in the skin of the patients who suffer from chronic inflammatory diseases as Discoid Lupus Erythematosus (DLE), Lichen planus, Lichen sclerosis, Lupus vulgaris, and dystrophic Epidermolysis Bullosa (EB) [5]. Age, UV radiation exposure, ethnicity, skin phototype, and immunocompromised can be known as classical risk factors for CSCC [6]. There are various etiologies considered for CSCC as predisposition determinants including host-derived immunity, environmental, genetic, and viral agents (HPV). From exterior agent’s views, there are different factors with a deteriorating role in CSCC pathogenesis such as UV rays, X-rays, arsenic compounds, and other chemical products [7]. The main cause of CSCC is UV rays with a mutagenic impact that is exacerbated by increasing latitudes [2,8]. Seemingly, in the tumor microenvironment, some agents have been discovered, acting as agitator increasing latitudes [2,8]. Inflammation, pro-inflammatory cytokines network, growth, and proliferation of keratinocytes can also be cited as etiologies for CSCC [17,18]. According to available reports, various cytokines constitute IFN-γ, IL-6, GM-CSF, and TGF-β are involved in the proliferation of skin keratinocytes and the pathogenesis of CSCC [19]. In the past, surgery was one of the most common therapeutic methods that have been noticeably used [20]. Also, different cytokines and medications have been used for treatment such as IL-24, later [10]. Fresolimumab is an anti-TGF-β monoclonal antibody, an immune suppressor, as another therapeutic method that is enumerated for CSCC [21]. All in all, identification of cytokine profile in the tumor microenvironment, their fluctuation, and changing is expected to facilitate CSCC diagnosis and treatment. Previous therapeutic methods faced with failure and have not been effective. Whereas optimistic, integrative and comprehensive methods have emerged and proposed cell and gene therapy by stem cells and vectors to revolutionize the existing therapeutic strategies [22-23]. To sum up, according to the mentioned cellular and molecular interactions involved in CSCC immunopathogenesis, authors optimistically offer gene and cell-based immunotherapeutic interventions using MSCs and IL-25 gene for physicians and specialists to accredit their orders for CSCC control and open glistening horizons to patients’ lifestyle.

IL-25 Signaling Pathway

IL-25 (IL-17E) is one of the IL-17 family members that is secreted by Type 2 helper T cell (TH2) and mast cells [21]. It is worth mentioning that the IL-17E isofrom of the IL-17 family is undetectable in non-lesional skin but it is plainly soared in lesional and psoriatic skin [24]. IL-17RB and IL-17RA are functional subunits of the IL-25 receptor that impose and trigger its immunobiological effects by this receptor [25-26]. MBP+ eosinophils, tryptase+ mast cells, and CD31+ endothelial cells are expression-in-cell sources of IL-25 and its receptor in bronchial submucosa and dermis. Hence, IL-25 can be placed at the central point of pro-inflammatory mediators released by affecting eosinophils [27]. Chromosome 14 (14q 11-12) is the genomic position of IL-25. IL-25 binds to its receptors and induces activation of FADD and TRADD complexes, then causes caspase-mediated apoptosis by activation of caspases 8 and 3 and subsequently, induces apoptosis [28]. Also, IL-25 can be effective in different immunological conditions such as parasitic helminths infection [29], asthmatic respiratory disorders [30], atopic dermatitis, [31] and different kinds of tumors [32]. Regarding to recent studies and findings, IL-25 represents caspase-mediated apoptotic effects on pancreatic and breast tumors and devastates tumoral mass without affecting non-tumoral cells [28,33] (Figure 1).
IL-25 binding to its receptors, FADD and TRADD complexes activation, caspases 8 and 3 activation, caspase-mediated apoptosis. B) Angiogenesis and tumor proliferation by attenuation of apoptosis, metastasis and by stimulation of different agents (such as Basic Fibroblast Growth Factor (bFGF), Hematopoietic Growth Factor (HGF), Transforming Growth Factor beta-1 (TGF-β1), Vascular Endothelial Growth Factor and Interlukine-8 (IL-8)).

The Hypothesis

Due to uncompleted recovery by previous therapeutic methods, this hypothesis aims to investigate the pro-apoptotic effects of IL-25 gene delivery by MSCs for CSCC immunotherapy. IL-25 has been introduced as a novel cytokine having the potential to be a significant target for novel therapies in different cancers, respiratory airways allergy, lung tissue disorders, parasitic and infectious disease [29-32]. Different stem cells such as Hematopoietic Stem Cells (HSCs), Cancer Stem Cells (CSCs) [11], Chimeric Antigen Receptor (CAR) T cells [34-35], MSCs [36], and various vectors can be recruited for this kind of gene and cell-based immunotherapies. According to current studies [37], cell and gene-based immunotherapies using MSCs and different vehicles are known as a novel approach in cancer treatment. MSCs are responsible for growth, wound healing, and cell replacement during pathological conditions. So they have a major role in repairing tissue damage and degenerative diseases that makes them a perfect vector for keeping antitumor factors [22]. MSCs seem to play an anti-tumoral role via down-regulation of anti-apoptotic factor (Bcl-2) and the PI-3K/AKT pathway inhibition [38]. MSCs have anti-tumoral features and potentially immunosuppressive side effects that help them to regulate the immune system, inflammation and have pathologic and therapeutic role in the tumor microenvironment [39-40]. This type of stem cells is easy to culture, manipulate and isolate in ex vivo culture. Viral and non-viral vectors are recommended in gene and cell-based therapy of cancer [41-42]. Different types of viruses have been modified in gene therapy applications including Retrovirus, Adenovirus, Adeno-Associated Virus (AAV), Lentivirus, and Herpes Simplex Virus (HSV) [43-44]. We need a vector without causing immune or genotoxicity properties. AAV is a DNA virus nonpathogenic vector with respect to safety. It can be used in clinical applications as a suitable vector for gene therapy goals [22,45-47]. AAV can be used for delivering therapeutic transgenes in different categories: cytotoxic or suicide genes, angiogenesis genes, tumor suppression, cytokines for stimulating the immune system, and anti-tumor gene [48]. Interleukins are key mediators for regulating inflammatory and immune responses in the cancer microenvironment and they can be beneficial therapeutic factors by using MSCs as delivery vehicles and AAVs as vectors for targeted immune gene and cell therapy of cancer [37]. According to the principles mentioned, tolerance/resistance to apoptosis is one of the pathogenesis basis in CSCC which can be a progressive pathway to recovery and treatment of the disease. Given that, IL-25 also has pro-apoptotic properties, many lines of evidence show that this cytokine can be valuable and effective in CSCC improvement and helping in the treatment of this kind of tumor. The overall purpose of this study is to provide a therapeutic idea for CSCC healing using IL-25, as the IL-25 gene is injected to a Dimethylbenz[a]Anthracene/12-o-Tetradecanoylphorol-13-Acetate (DMBA/TPA) [18] CSCC animal model using gene therapy and cell therapy through genetically modified MSCs recruitment. This makes the malignant cells more exposed to IL-25 and induces apoptosis in tumor cells.

Evaluation of hypothesis

According to cases pointed earlier, we propose the following levels to test and assess the hypothesis (Figure 2).

1. Initially induction of syngeneic DMBA/TPA CSCC mouse model, the separation of MSCs from the bone marrow of mice model, and Adeno-Associated Virus (as a vector) for transduction of the IL-25 gene into the mentioned model. It is of high prominence to mention that all of the injections are done subcutaneously.

2. Control group, containing 10 syngeneic DMBA/TPA CSCC animal models (receive no injections).

3. Case groups, characterization of 30 mice into three categories, as below: 3a) Transduction of MSCs by IL-25 gene delivery into the First case group (n=10). 3b) AAV– IL-25 gene delivery into Second case group (n=10).

3c. MSCs and empty AAV vector into Third case group (n=10).

4. Isolation of MSCs: carrying out from DMBA/TPA mouse model bone marrow and subsequent culture [49].

5. Serum IL-25 levels detection by Enzyme-Linked Immunosorbent Assay (ELISA).

5a. Detecting apoptotic DNA fragmentation by Terminal deoxyribonucleotyl Transferase UTP Nick End Labeling (TUNEL) assay [33].

5b. Measuring the expression levels of cell surface markers by Flow cytomtery, intracellular molecules and intracytoplasmic cytokines using fluorescence-conjugated antibodies.

5c. IL-25 gene expression by Real-time Polymerase Chain Reaction (PCR).

6. Transfusion of mouse’s modified gene MSC to own bloodstream.
Discussion & conclusions

In this hypothesis, given the importance of CSCC, the authors have attempted to provide a better, more effective and comprehensive treatment with the least complications and side effects. Previous treatments such as chemotherapy, radiotherapy, monoclonal antibody and other methods did not have enough constructive effects on CSCC improvement. According to the molecular aspects related to the tumor microenvironment, defects in apoptosis mechanisms play important and deleterious roles in tumor pathogenesis, so induction of apoptosis processes can be a reasonable method for treatment of this malignancy [5]. IL-25 (IL-17E), via binding to its receptor, can induce apoptosis in cancerous cells without affecting nonmalignant cells. Also, IL-25/IL-25R signaling can induce a pro-inflammatory response in lung fibroblastic tissues. Pro-apoptotic impacts of IL-25 have been expressed in breast and pancreatic cancer by triggering activation of FADD and TRADD complex, then caspases 8 and 3 activations have occurred [28,33]. Understanding of the immunological and molecular mechanisms of cancer microenvironment could be efficient for novel perspectives on gene and cell-based immunotherapies that have been an appropriate strategy for cancer and various kinds of disorders such as Psoriasis [47,50-52], Behcet’s disease [53], Hodgkin’s lymphoma [54], Pancreatic cancer [33], Prostate cancer [55] and Thyroid cancer [56]. There are different kinds of studies that have investigated cytokines effects on cancerous cells. IL-24 is one of the cytokines that has a pro-apoptotic effect on solid cancer cells specially CSCC. Its intra-tumoral injection with adenosine vector was effective in previous studies [9-10]. IL-25 (IL-17E) had a pro-apoptotic role in the breast cancer microenvironment and affected tumor cells without affecting nonmalignant cells [28] also it is an agent that has antitumor efficacy in melanoma, colon, lung, and pancreatic cancer [57]. Biologic immunotherapy and immunomodulatory therapy can be an effective approach for treating CSCC because of it’s immunopathology mechanisms. Viruses commonly used in cancer gene therapies, AAVs have high transduction efficiency and Low immunogenicity [48]. They can be an appropriate and nonpathogenic vector for transducing MSCs by the IL-25 gene. Because of the multipotent, bystander and immunomodulatory properties of MSCs and their ability to repairing tissue damages and degenerative disease [22,58]. Recently the application of stem cell therapy and particularly MSCs transplantation for cancer therapy has become the center of focus among the investigations [37,59], it is hoped that these cells have an effective role in preventing the progression of CSCC by being injected in the CSCC mice model. The absence of apoptosis is one of the important mechanisms of cancerous cells. This challenge encourages basic clinical scientists to new directions as the appropriate generation of immunotherapeutic approaches. It seems that further investigations on IL-25 with its pro-apoptotic effects and IL-25/IL-25R signaling, may provide novel and promising therapeutic opportunities for the treatment of CSCC, be a reliance for oncologist’s concerns diminution and also impose less expenditure for patients.

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Authorship Contributions

This hypothetic study couldn’t be completed unless unsparring efforts and technical guides of Abdolreza Esmaeilzadeh for conceptualization qualifying, project administration, study design, scientific writing, theoretical and academic peer reviewing, definitive approval of the final manuscript, precious guides of Nazila Bahmaie for comprehensive advice on manuscript grammatically and Azita Mohammadzadeh for main conceptualization and collecting data, conclusive literature review, images designation, normative writing (last original drafts preparation). All authors have approved the final version of the article.

Ethical Consideration

Ethical issues (including plagiarism, double publication) have been completely observed by the authors.

References

15. Ratushny V, Gober MD, Hick R, Ridky TW, Seykora JT. From kera-


58. Esmaeilzadeh A, Reyhani E, Bahmaie N. Immunobiology of Dental Tissue-Derived Stem Cells; As a Potentiated Candidate for Cell Therapy. trials. 3: 28-29.