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Cancer Stem Cells derived from mouse iPS cells

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Abstract

The cancer stem cells (CSCs) capable of continuous proliferation and self-renewal are considered to play significant roles in oncogenesis, tumor growth, metastasis and cancer recurrence. CSCs should be derived from normal stem cells affected by the tumor microenvironment although the mechanism of development is not clear yet. On the other hand, induced pluripotent stem cells (iPSCs) as well as embryonic stem cells are considered to be induced into progenitor cells, which differentiate into various normal phenotypes depending on the normal niche. We hypothesized that CSCs could be derived from iPSCs in the conditioned culture medium of cancer cell lines, which might be a mimic of carcinoma microenvironment. In this study, we employed Nanog mouse iPSCs, in which GFP gene has been inserted into the 5' untranslated region of the Nanog gene. As a result, the cells treated with the conditioned medium for 4 weeks exhibited a high tumorigenicity *in vivo* with a capacity of self-renewal and the expression of markers associated with stem cell properties and an undifferentiated state. The cells efficiently formed spheroids expressing GFP in suspension culture, vasculogenic tubes in the presence of type IV collagen *in vitro* and exhibited extensive angiogenesis *in vivo*, which was confirmed by magnetic resonance imaging and by histochemical analysis. From the tumors the CSCs expressing GFP were isolated as spheroid forming cells in the primary culture. These cells showed almost the same characters as those found in the originally transplanted CSCs. Furthermore, the CSCs exhibited extensive metastasis to lung when injected into tail vein. Thus we concluded that a model of CSCs was originally developed using mouse iPSCs and proposed the conditioned culture medium might perform as niche for producing CSCs. It is worthwhile noticing that the mouse iPSCs co-cultured with cancer-derived cells did not form malignant tumors *in vivo*. This implies that cell-to-cell contact may have inhibitory effect on the conversion of mouse iPSCs into CSCs. The tumor microenvironment, which converted mouse iPSCs to CSCs, as well as the heterogeneity in tumor tissues, will be further discussed.

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Peptide Vaccines for Cancer

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Abstract

Background:

In general, the preferable characteristic of the target molecules for development of cancer vaccines are high immunogenicity, very common expression in cancer cells, specific expression in cancer cells and essential molecules for cell survival (to avoid loss of expression). We previously reported that three novel HLA-A24-restricted immunodominant peptides, which were derived from three different oncoantigens, TTK, LY6K, and IMP-3,were promising targets for cancer vaccination for esophageal squamous cell carcinoma (ESCC)patients. Then, we had performed a phase I clinical trial using three HLA-A24-binding peptides and the results had been shown to be promising for ESCC. Therefore, we further performed a multicenter, non-randomized phase II clinical trial.

Patients and Methods:

Sixty ESCC patients were enrolled to evaluate OS, PFS, immunological response employing ELISPOT and pentamer assays. Each of the three peptides was administered with IFA weekly. All patients received the vaccination without knowing an HLA-A type, and the HLA types were key-opened at the analysis point. Hence, the endpoints were set to evaluate differences between HLA-A*2402-positive (24(+)) and -negative (24(-)) groups.

Results:

The OS in the 24 (+) group (n=35) tended to be better than that in the 24(-) group (n=25) (MST 4.6 vs. 2.6 month, respectively, p = 0.121), although the difference was not statistically significant. However, the PFS in the 24(+) group was significantly better than that in the 24(-) group (p = 0.032). In the 24(+) group, ELISPOT assay indicated that the LY6K-, TTK-, and IMP3-specific CTL responses were observed after the vaccination in 63%, 45%, and 60% of the 24(+) group, respectively. The patients having LY6K-, TTK-, and IMP3-specific CTL responses revealed the better OS than those not having CTL induction, respectively. The patients showing the CTL induction for multiple peptides have better clinical responses.

Conclusion:

The immune response induced by the vaccination could make the prognosis better for advanced ESCC patients.

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Combining AIET with chemotherapy - lessons learnt from our experience

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Abstract

Breast cancer is the most common invasive cancer in women and as per the data in 2008, this deadly cancer was responsible for 458,503 deaths worldwide in 2008 [1]. Several researches are on-going for identifying therapeutic strategies for breast cancer. Breast cancer biology is complex and breast cancer stem cells that are often resistant to conventional therapies like chemotherapy [2] increases the complexity as it has been reported that at even early stages of the disease, a portion of the breast cancer cells may have eloped to the bone marrow facilitated by the mesenchymal stem cells [3] and remain dormant becoming active later thereby causing recurrence or advancement of the disease. Natural Killer (NK) cell based Autologous Immune Enhancement Therapy (AIET) which has been administered for different types of cancers [4,5] represents a potential option, as NK cells being a part of innate immunity help in tackling circulating cancer cells [6] and cancer stem cells [7] thereby helping to prevent metastasis. Herein we report our experience of NK cell based AIET in a patient of stage III A breast cancer (inflammatory type) diagnosed three months post-partum. A 29 year old female with history of pain and tenderness in the left breast post-partum was investigated in October 2012 and the investigations revealed the presence of infiltrating ductal carcinoma (pT3 N2a Mx- Stage III A) (T4bN2M0) and the cancer was ER positive, PR negative, Her2neu negative, Ki67 positive (86% of the tumour cells) and EGFR, Cytokeratin 5 negative. The patient underwent three cycles of pre-operative chemotherapy (from October 2012 to December 2012) using Doxorubicin, Docetaxel and Cyclophosphamide followed by left modified radical mastectomy (December 2012) and then three cycles of post-operative chemotherapy (from January to February 2013). The patient simultaneously underwent 12 transfusions of NK cell based AIET from November 2012 to February 2013 planned in accordance with the chemotherapy cycles. Approximately 200-220 ml of peripheral blood (PB) was withdrawn for the first three cycles (three transfusions in one cycle – nine transfusions in total) and then for the 10th transfusion, only 40 ml of PB was withdrawn as the patient's general health condition was low. 185 ml of PB was withdrawn for the 11th and 12th transfusions. For each AIET transfusion, the NK cells isolated from peripheral blood mononuclear cells (PBMNCSs) were culture-expanded in vitro based on earlier described protocols [8, 9] for 10-12 days before being infused to the patient. Chemotherapy has earlier been reported to cause a decline in NK cell number and function [10]. It was observed in the present case that, with progressively increasing number of chemotherapy cycles, there was a gradual decline in the in vitro growth expansion of the NK cells, though the number of NK cells isolated from the peripheral blood remained fairly constant. After AIET and chemotherapy, the patient underwent radiotherapy with 5400cGy in 27 fractions. The patient is under follow-up. The decrease in NK cell expansion potential with progressive chemotherapy implies a weakened immune system after chemotherapy. This experience could be an eye opener to understand the potential weakness of the immune system with the present therapies enable us undertake researches to come out with more targeted therapies which shall not compromise the immune system thereby plugging the loopholes to prevent metastases and to yield a better prognosis.

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'An eye, for eyes - mission' - From dream to reality

Parikumar P[1], Haraguchi K[2], Senthilkumar R[3], Abraham S[4,5]

Abstract

Introduction:

Corneal transplantation has been in routine practice to treat corneal endothelial diseases like Bullous Keratopathy, in which either the whole cornea or the partial cornea (the endothelium alone) is transplanted from the cadaver donor to the recipient with the endothelial disease [1]. In whole corneal or partial corneal transplant one cadaver donor's cornea can be used to treat one recipient cornea only, which leads to a huge global shortage of donor corneas [2]. At this juncture Yokoo et al isolated and expanded corneal endothelial precursors using the sphere forming assay in vitro [3] and demonstrated the in vivo transplantation of these corneal endothelial precursors in a rabbit model of bullous keratopathy [4]. Following this, we studied the transportation of cadaver donor derived corneal endothelial tissue (CET) from human cadaver donors in a thermoreversible gelation polymer (TGP) (4) based transportation cocktail without cool preservation and demonstrated the viability of human corneal endothelial precursor (HCEP) cells isolated from these CETs even after 72 hours of transportation without cool preservation [5]. This was done to suit the conditions existing in developing nations like India where hospitals might be located far from eye banks and maintaining cold chain preservation is relatively difficult. Further, these HCEPs were expanded in vitro using a polymer based expansion protocol [5]. This was the first step in the realisation of the dream of 'Eye for eyes' in a manner suitable for Indian conditions.

Hurdle faced in Clinical Translation and its solution:

After HCEP transplantation, the eye balls need to be fixed 24- 36 hours facing down, to facilitate the gravity-assisted settling of the cells injected into the anterior chamber on to the endothelium which is possible in animals but difficult in humans. To overcome this hurdle, we used the nanocomposite gel sheet (D25-NC gel sheet) developed by Haraguchi [6] as a supporting material to support the HCEP cells during transplantation and the HCEP transplantation using this NC gel sheet was successfully demonstrated in a cadaver bovine's eye cornea [7]. Thus the second step in the 'Eye for eyes' mission was accomplished.

The Pilot Clinical study:

The study was undertaken in three patients, two suffering from bullous keratopathy and one patient with congenital corneal dystrophy after proper informed consent. The right eye was affected in each patient and the transplantation of HCEP cells were done in these right eyes. 6 x 104 HCEP cells isolated from one human cadaver donor cornea were expanded using the polymer based protocol [5] for 26 days. After expansion, the 5x105 HCEP cells obtained were divided into three portions. Using the NC gel sheet as supporting material approximately 1.6 x 105 HCEP cells suspended in saline were infused into the anterior chamber between the recipient endothelium and the NC gel sheets in each patient. The extension arms of the NC gel sheets were buried under the conjunctiva and sutured. All the three patients were kept under observation and examined using slit lamp at regular intervals. After three days the NC gel sheets were removed done under topical anaesthesia and sent for microscopic examination. There were no HCEP cells attached to the NC gel sheet, removed three days after transplantation. In all the three patients, on post-operative Day 11, the cornea became clear with no evidence of bullae. The patients are under long term follow-up.

Conclusion:

The eye for eyes concept has become a reality by combining the strengths of clinical expertise with cell culture and synthetic material technology in a manner which is easy to reproduce both in the laboratory and clinically.

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Role of tissue engineered buccal mucosa for treatment of urethral stricture

Vaddi S[1], Godala C[1], Reddy V[1], Senthilkumar R[2], Srinivasan T[2], Reena H[2], Preethy S[2], Abraham S[3, 4]

Abstract

Introduction

Cell based therapies in Urology:

Cell based therapy for tissue engineering in urology, like in other branches of medicine uses the principles of cell transplantation, materials science, and biomedical engineering to develop biologic substitutes that can restore and maintain function of the damaged or lost genitourinary organs. Most current strategies for tissue engineering depend on a sample of autologous cells from the diseased organ of the host. However in cases where primary autologous cells cannot be expanded, pluripotent stem cells are an ideal source. Biomaterials play a major role in genitourinary tissue engineering. They are used to replace biologic and mechanical functions of the native extracellular matrix. Three classes of biomaterials have been used for the engineering of genitourinary tissues: naturally derived materials, such as collagen and alginate; acellular tissue matrices, such as bladder submucosa and synthetic polymers, such as polyglycolic acid [1]. A lot of research is ongoing in urethral regeneration by tissue engineering and cell based therapy. Tubularized collagen matrices seeded with autologous cells are used to regenerate the urethra [2]. Urinary Bladder reconstruction is possible with bladder shaped biodegradable scaffold seeded with autologous urothelial cells and smooth muscle cells [3]. Ureteral acellular tubular grafts have been used to replace ureteral loss but with poor functional results [4]. Cell-seeded biodegradable polymer scaffolds have been used with more success to reconstruct ureteral tissues [3]. The kidney is the most challenging organ in the genitourinary system to reconstruct because of its complex structure and function. Cell based therapies are used for creation of functional renal structures *in vivo*. Renal tubular cells have been harvested, expanded in culture and seeded on to a tubular device to function as nephron [5]. The expansion of this system to larger three-dimensional structures is the next challenge awaiting researchers in the urogenital tissue engineering field. Genitalia reconstruction is also possible with cell therapy. Engineered penile prosthesis can be reconstructed by culturing autologous chondrocytes which are seeded onto a Poly-glycolic acid scaffold and then implanting the scaffold into the corporal space of penis [6]. Microencapsulated Leydig cells in animal studies have been used to replace or supplement testosterone in testicular failure [7]. Cell therapy techniques are also used for treatment of urinary incontinence, vesicoureteric reflux by injecting cultured myoblasts or adipocytes [5]. The major limitation in engineering solid organs is the vascularisation of the regenerated tissue. Recent developments in angiogenesis research [8] may provide answer to this complex problem and accomplish the goal. Most of the research to date in urological tissue engineering is done in animals. Before these engineering techniques can be applied to humans, further studies need to be performed.

Buccal Mucosal Epithelium for repair of the short segment urethral stricture:

Urethral stricture is the narrowing of the lumen of the urethra which occurs as a terminal event secondary to many etiologies. Patients present with difficulty in voiding urine. There are endoscopic and open surgical reconstructive procedures to treat this disorder. Endoscopic treatment is often temporary and eventually results in recurrence of the disease. Many open surgical procedures have been described but none of the procedures offer permanent cure. The use of buccal mucosal grafts for stricture repair is in practice [9,10] with considerable success. However the donor site morbidity and complications like stricture recurrence with the present techniques [11,12] warrant the advent of novel techniques. The use of buccal mucosal cells which can be obtained by harvesting a 2mm x 2mm tissue bit compared to that of 5-6cm tissue usually harvested in conventional techniques for a graft and the culture-expansion of these cells in a suitable *in vitro* scaffold which can also act as a substrate after transplantation *in vivo* for optimal repair provides a viable

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option. Preliminary results of the application of autologous human buccal epithelial cells expanded & encapsulated in a nanopolymer scaffold after confirmation of their phenotype and genotype, in a male patient with inflammatory urethral stricture have been encouraging. This approach we have named as the Buccal Epithelium Expanded & encapsulated in Scaffold - Hybrid Approach to Urethral Stricture (BEES-HAUS) and is seemingly a promising one and further studies are needed for its validation.

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