Nasopharyngeal Carcinoma Stem Cells

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Nasopharyngeal Carcinoma (NPC) frequently occurs in the epithelial lining of the nasopharynx. It is a common cancer occurring in Southern China and South East Asia, while this disease is comparatively rare in most other parts of the world. Development of the disease is closely related to genetic, viral, environmental and dietary factors. Among these, Epstein Barr Virus infection is consistently associated with NPC. Cancer stem cells (CSC), a subset of cancer cells within a tumor having the ability to self renew and differentiate, are recently reported to be closely related with recurrence of tumours. In comparison, using the ability of exclusion of the DNA dye Hoechst 33342 as the parameter, side population (SP) cells can be isolated from the heterogeneous cell populations in tumour mass. SP cells may share characteristics of CSCs, both being enriched for tumor initiating capacity, and resistance to chemotherapy and radiotherapy. Although SP cells and CSCs were recently reported in NPC cell lines, so far no solid biomarkers were identified. NPC is often diagnosed late due to its deep location and no specificity of symptoms when the disease is in its early stage. Thus, there is a need to find sensitive biomarkers for an early diagnosis and even treatment of NPC. Screening strategies have being developed using the genomics, proteomics, immunology, miRNA and bioinformatics for discovery of molecular biomarkers.

Key words: Nasopharyngeal carcinoma, cancer stem cells, side population cells, biomarker

Introduction

The Nasopharyngeal carcinoma (NPC) is a cancer arising from mucosal epithelial cells that covers the surface of the nasopharynx [1]. NPC differs significantly form other cancers of the head and neck in its occurrence, causes, clinical behavior and treatment [2]. NPC is generally a rare cancer world widely, with an average of 80,000 new NPC cases recorded per year (i.e. 0.7% of all cancers), males are more frequently affected than females, with the male to female ratio being approximately 2.3:1 [3]. Comparatively, NPC is relatively common cancer in Chinese or Asia ancestry. It poses one of the serious health problems in southern China, where an annual incidence of more than 20 cases per 100,000 is reported [4].

NPC Clinical Syndromes and Classification

Signs and symptoms of NPC at presentation include painless and enlarged cervical lymph nodes, nasal obstruction, epistaxis, diminished hearing, tinnitus, recurrent otitis media, cranial nerve dysfunction, sore throat and headache [4]. The World Health Organization (WHO) classifies NPC into three types. Type I or Squamous cell carcinoma (SCC), or keratinizing squamous cell carcinoma is seen in 25% of NPC. Type I tumor produces keratin and demonstrate the presence of intracellular bridges when observed under electron microscope. Type II or non-keratinizing carcinoma (NKC) occupies approximately 20% of NPC. These tumor cells demonstrate a range of cellular morphologies, from mature to anaplastic. Type III or undifferentiated carcinoma (UC) is observed in 55% of NPC. The tumor cells have oval or round vesicular nuclei and prominent nucleoli. Besides,
the cell margins are indistinct, and the tumour exhibits a syncytial rather than pavemented appearance [5].

The NPC Etiology

Previous studies revealed that several factors are involved in the pathogenesis of NPC including genetics, virology, and environmental and dietary factors combined in a multistep process [4]. Several linkage analysis reports suggested the association of susceptibility HLA haplotype with NPC development, in Chinese population an increased risk for NPC is with HLA A*0207 [6]. Genetic susceptibility loci linked to NPC development have been reported on chromosomes 3 and 4 [7, 8]. Recent linkage studies from Greenland and Denmark further indicated an increased risk for infectious agent related cancers including salivary, cervical and gastric cancers was observed among families with history of NPC [9].

Epstein Barr virus (EBV) is a major risk factor for the development of NPC, where it is constantly detected in the NPC patients form regions with high and low incidence, specific EBV genes are consistently expressed within the NPC tumors and in early dysplastic lesions, the viral proteins latent membrane protein 1 and 2 having profound effects on cellular gene expression and cellular growth, result in highly invasive and malignant growth of NPC tumors [10]. EBV-encoded RNA signals have been shown by in situ hybridization, to be present in nearly all NPC tumor cells and absent in adjacent normal tissue, expect perhaps for a few scattered lymphoid cells [11]. The traditional foods of Southern Chinese, such as Cantonese style salted fish and other preserved foods containing volatile nitrosamines are an important carcinogenic factor for NPC [12].

Cancer Stem Cells in General

Human malignancies consist of two functionally distinct cell types: Cancer stem cells (CSCs) and Non-self-renewing progeny cells[13]. CSCs may arise from self renewing normal stem cells which are transformed by dysregulation of a self renewal pathway [14, 15]. Basically, CSCs are a subset of tumor cells that have the ability to self-renew and generate the diverse cells to form the tumor [14, 16, and 17]. The self renewal properties of the CSCs are the driving force of tumorgenesis [14]. The first evidence for CSCs came from acute myeloid leukemia (AML) in which a rare subset comprising 0.01-1% of the total population can induce leukemia when transplanted in to immunodeficient mice [14, 18]. So far only two general approaches have been adopted to identify and characterize CSCs. They are: 1. Molecular markers and 2. Side population cells isolated from tumors [19].

CSCs Bomarkers

Increasing evidences exist for CSCs identified in a variety of solid tumors. Recent researches have identified the existence of CSCs from tumours of many organs, including brain, breast, colon, pancreas, liver, and other tissues [20]. CSCs were identified by using different cell surface markers shown in Table 1. Generally, tumors appear to vary in the percentage of CSCs that they contain, with reported values ranging from 0.03 % colon tumour [21] to nearly 100% Melanoma tumour [22]. Several human solid tumors have been studied utilizing NOD/SCID mice (Nonobese diabetic/severe combined immunodeficient mice) as recipients for tumor xenografts. This approach has been applied in brain [23], colon [24], head and neck [25], and pancreatic tumours [26]. CSCs are thought to be involved in cancer recurrence owing to their tumorigenic properties and supposed resistance to many conventional therapies [27]. CD133+ cells in fresh glioblastoma specimens or glioma xenografts irradiated in vivo were more resistant to ionizing irradiation than CD133- cells. Thus, an expansion in the subset was found following irradiation both in vitro and in vivo. Notably they observed that CD133+ cells preferentially activated the DNA damage checkpoint response more effectively than CD133- cells. CSC cell population appears to have evolved a more efficient DNA damage repair system than the bulk of the tumour, conferring resistance to radiation treatment [28].

The mechanisms underlying drug resistance are poorly understood. Various stem cells often express higher levels of drug-resistance proteins such as ATP-binding cassette half-transporter proteins (ABCG2 and ABCG5) and multidrug resistance protein 1 (mDR1) transporters, and augmented levels of these in CSCs may contribute to the refractoriness of metastatic cancer to chemotherapy [29]. Drugs that seem to specifically target and eliminate CSCs in patients were recently reported. For example, parthenolide and rapamycin appear to kill CSCs in Acute Myeloid Leukaemia but not in normal hematopoietic stem cells [30, 31]. Temozolomide also preferentially eliminates CSCs in glioblastoma [32] and brain CSCs treated with bevacizumab have decreased tumorigenicity [33]. Although there are so many
Side Population Cells in Human Cancers

Side population (SP) analysis could be used to identify CSCs [34]. SP cells represent only a small fraction of the whole cell population; they appear to be enriched in stem cells. Thus, they could provide a useful tool and a readily accessible source for cancer stem cell studies [35]. SP cells were first isolated from murine bone marrow via staining with the vital DNA dye Hoechst 33342, followed by analysis and purification using the fluorescence-activated cell sorter (FACS) [36]. Bone marrow SP cells appear Hoechst 33342 dull or low compared to the Main Population (MP) of cells, which exhibit Hoechst 33342 brighter staining. The reduced Hoechst 33342 staining of bone marrow SP cells was demonstrated to be due to their capacity to efflux Hoechst 33342, mediated by the ABCG2/bcrp1 transporter [37, 38].

Recently, SP cells were identified in numerous tumor tissues such as liver [39], brain [40], heart [41], skeletal muscle [42], glioblastoma [43], head and neck [44], lung [45], mammary gland [46], skin [47], and NPC [48]. SP cells share many stem cell characteristics, including a long life span, relative quiescence, and resistance to drugs and toxins through ABC transporter expression. Compared with the bulk of non-tumorigenic cancer cells, SP cells at a lower cell number have the ability to form tumor after transplantation. Because they are resistant to chemotherapy and radiotherapy, they may contribute to tumor relapse even after most non-tumorigenic cells are destroyed [16]. SP cell are isolated from hepatocellular [49], gastric [50], lung [51] and nasopharyngeal carcinoma [48] cell lines were highly enriched for the capacity to initiate tumor formation when xenografted in to NOD/SCID mice.

SP cells have increased expression of genes that are believed to involve the regulation of stem cell function compared to non SP cells. Using microarray analysis and validation with RT-PCR analysis, SP cells from breast cancer [52], hepatocellular [49], gastrointestinal [50] and thyroid [53] cancer cell lines were unregulated in the expression of the ABCG2 transporters when compared with non SP cells. In breast carcinoma cell line (MCF-7), SP cells show an increase in the expression of genes involved in the cell cycle regulation, including EXT1, INHBA, and CCNT2, than non SP cells. SP cells were also shown to have more cells in G1/Go phases than non SP cells in breast cancer [54]. Differential expression analysis of cytokines expression indicated cytokine 19 was more highly expressed in fresh SP cells than non SP cells in Nasopharyngeal carcinoma [48].

Molecular Biomarkers in NPC

As NPC is a complex disease caused by multiple factors and developed in a multi-step process of carcinogenesis, together with its deep location and vague symptoms, it is generally diagnosed at a later stage in disease development [55]. Elevated RNase activity has previously been described in the circulation of cancer patients, and NPC was found to be associated with disturbances in the integrity of cell-free circulating RNA. Measurement of plasma RNA integrity may serve as a useful marker for the diagnosis and monitoring of NPC [56]. CDH13 (encoded a cell adhesion molecule H-cadherin) promoter is
aberrantly methylated in NPC both in vitro and in vivo, and promoter methylation plays a pivotal role in the silencing of H-cadherin expression. Furthermore, the high sensitivity (81%) and specificity (0% false positives) of detecting CDH13 methylation from nasopharyngeal swabs suggest it could be utilized as a tool for early diagnosis.[57] The NPC biomarkers currently identified by proteomics technology are shown in Table 2.

RASSF1A gene which is a tumor suppressor gene identified on 3p21.3 frequently inactivated by promoter hypermethylation in NPC. Investigated by high density oligonucleotide array, the expression of activin βE and Id2 in NPC were tightly regulated by RASSF1A. RASSF1A-induced repression of Id2 was mediated by the over expression of activin βE. The results suggested a novel RASSF1A pathway in which both activin βE and Id2 are involved [58]. Besides, KIAA1173 gene located at 3p22.1 is strongly expressed in normal nasopharyngeal mucosa epithelia but down regulated in NPC, which may also be associated with carcinogenesis of NPC [59]. Furthermore, polymorphism of nitrosamine metabolizing gene, CYP2A6, might play a crucial role in NPC susceptibility and CYP2A6 might be used as a risk marker for NPC [60]. Using oligonucleotide microarray analysis, THY1 gene located on 11q22-23 region was identified to be a candidate tumor suppressor gene significantly associated with metastatic NPC [61]. Another gene BLU/ZMYND10 which is one of the candidate tumor suppressor genes and mapped in the 3p21.3 critical region is also a candidate tumor suppressor gene for NPC. By quantitative RT-PCR, BLU/ZMYND10 is frequently downregulated in NPC cell lines (83%) and NPC biopsies (80%) [62]. The DLC-1 gene, located at the human chromosome region 8p22, is another tumor suppressor gene frequently deleted in NPC [63].

Recent evidences suggested that microRNA (miRNA) have major functions in the pathogenesis of tumor, around 50% of miRNAs are localized in cancer associated genome regions [64, 65]. The miRNA signatures have been proposed as a diagnostic and prognostic marker for various types of human cancers [66, 67]. Using Proteomics and microarray analyses, each miRNA may regulate the expression of hundreds of target genes [68, 69]. Recent studies using a miRNA target reverse screening method showed that miR-16 family triggers a cell cycle arrest by silencing multiple cell cycle regulatory genes simultaneously, rather than the individual target [70]. Several known oncogenic miRNAs including as mir-17-92 cluster and mir-155, are among the miRNAs up-regulated in NPC. Tumor suppressive miRNAs including the mir-34 family, mir-143, and mir-145, are significantly down-regulated in NPC, these results indicated that these down-regulated miRNAs may coordinately regulate several oncogenic pathways in NPC [71].

**Conclusion**

NPC is very difficult to identify in early stages, because of the inaccessibility of the anatomic site. The purpose of studying CSCs in NPC is to develop strategies for effective cancer prevention, early diagnosis and treatment. The death rates for NPC will be expected to reduce due to advances in early detection and prevention. Signaling pathways and cell surface markers utilized by NPC CSCs can be targeted to block the cancer progression at each stage. CSCs are inherently resistance to a variety of chemical and radiotherapies. These cells may have genetic mutations that make them resistant to damage from chemotherapy and targeted therapy. They may be able to repair DNA damage more efficiently than normal cells. Based on these characteristics, NPC CSCs need to be eradicated in order to provide long term disease-free survival. Identification of potential markers using technologies such as genomics, proteomics, immunology, miRNA and bioinformatics will play very

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<th>Biomarkers</th>
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<tr>
<td>CK19, EBP1, Rho-GDI-2</td>
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<tr>
<td>Stathmin, 14-3-3s, Annexin A1</td>
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<td>Ceruloplasmin</td>
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<td>Fibronectin, Mac-2 BP, PAI-1</td>
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<tr>
<td>Fragment of interalpha-trypsin inhibitor precursor, platelet factor-4</td>
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<tr>
<td>Serum amyloid A protein (11 800 Da) and 11 600 Da</td>
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<tr>
<td>Complement C3f fragment (2020 Da) and 4635 Da</td>
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<tr>
<td>4053, 5885, 4072, 5798, 4209, 8689, 2382, 9357, 2221, 4230, and 5901 Da</td>
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<tr>
<td>6692, 6811, 6862, 7979, 9176 and 10272 Da</td>
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important roles in NPC CSCs diagnosis.

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References


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鼻咽癌發生於鼻咽部的上皮細胞。此疾病常見於中國南部及東南亞，較少見於世界其他地方。許多遺傳、病毒、環境及飲食因素皆與鼻咽癌生成有關。其中EB病毒與致癌關係甚為密切。癌幹細胞指一群俱自我新生及分化能力的癌細胞，與癌症複發有關。利用Hoechst 33342 DNA染料，次細胞群癌細胞可由癌細胞株中分離出來，次細胞群癌細胞與癌幹細胞可能有共同特性。雖然具癌幹細胞特性之次細胞群癌細胞已由鼻咽癌細胞株中分離出來，但目前並無生物標記被報導。當務之急為以基因體、蛋白體、免疫學及生物資訊找出鼻咽癌幹細胞之生物標記，以促進診斷及治療效率。

關鍵詞：鼻咽癌、癌幹細胞、次細胞群癌細胞、生物標記

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