Dr. Poonam Gaur

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Status: Married

Educational Qualifications (Teaching Exp 3 yrs)

Examination Passed	Year	School /College	Board /University	Subjects	Percentage of Marks
PhD	2016	A.I.I.M.S	A.I.I.M.S	Biotechnology	**Degree Awarded**
Masters	2010	A.I.I.M.S	A.I.I.M.S	Recombinant DNA technology, Structural Biology, Bioinformatics, Cell Bio, Biochemistry, Microbiology, Bacterial and human genetics, Molecular bio, Immunology	**74.0%**
Bachelors	2008	Deshbandhu College	University of Delhi	Biochemistry, Cell Biology, Molecular Biology, Genetics, Physiology, Immunology, Physics, Chemistry, Maths & Statistics,	**79.8%**
12th	2004	D.P.S., R. K. Puram	C.B.S.E	Physics, Chemistry, Biology, Maths, English	**87.4%**
10th	2002	Navyug School	C.B.S.E	Hindi, Eng, Maths, Social Studies, Science	**88.2%**

Awards & Fellowships

- ✓ July 2010: Qualified ICMR-JRF award.
- ✓ June 2010: Qualified CSIR (JRF-NET) award with all India 058/0888 rank in Life Sciences.
- ✓ March 2010: Qualified GATE with all India rank 1023/10422, score 0382, marks 45.33/100
- ✓ Feb 2010: Qualified DBT-JRF (group A) award funded by DBT, Govt. Of India
- ✓ Dec 2009: Qualified CSIR (JRF-NET) award with all India rank 145/0918 in Life Sciences
- ✓ 2008-2010: Awarded fellowship by Department of Biotechnology, Govt. of India for the master's project entitled "Post graduate training program in Biotechnology with special application to biomedicine".
- ✓ 2008: Secured 11th position in the University of Delhi (Biochemistry) and 2nd in the college during graduation
- ✓ 2002: Received award of excellence in Hindi and certificate of merit for securing first position in standard X by Navyug School educational society.
- ✓ 2000: Awarded Scholarship by National Science Talent Hunt conducted by NDMC for securing first rank at state level in standard VII.
- ✓ 1996 : Awarded Scholarship by National Science Talent Hunt conducted by NDMC for securing sixth rank at state level in standard IV.
- ✓ Won prizes in inter school painting competitions, Quiz, music and sports.

Research Experience

Title: **Genetic Polymorphisms in Th2 cytokine genes** (**Masters Dissertation work)**

Duration*:* May 2009 – Jan 2010

Summary: We evaluated the association of selected single nucleotide polymorphisms (SNPs) in the promoter region of *IL4* [-589 T>C (rs2243250), -33 T>C (rs2070874)] and *IL6* [-174 G>C (rs1800795)] genes and exon 1 encoding leader sequence of *TGF-β1* [codon 10 (869 T/C::Leu/Pro), codon 25 (915 G/C::Arg/Pro)] gene with the development of tobacco-related oral squamous cell carcinoma (OSCC) in Asian Indians. The genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method in 140 OSCC patients and 120 healthy controls. We exploited Bioinformatics tools to identify potential SNPs in these genes and to design primers. Genomic DNA was isolated from peripheral blood using sodium-perchlorate method and subjected to PCR amplification using specific primers. The positively amplified samples after cross-checking were digested with particular restriction enzymes and the resultants were resolved by 3%

agarose gel or 8% PAGE (polyacrylamide gel) electrophoresis. Statistical analysis was performed using STATA/IC-11.2 software. Logistic regression analysis was done to assess the risk associated with these SNPs. All evaluated SNPs followed Hardy Weinberg equilibrium and haplotype analysis showed strong linkage disequillibrim between *IL4* and *IL6* SNPs. We demonstrated that these functional SNPs were associated with susceptibility of OSCC development and these may be exploited as potential prognostic marker.

Title: **Phenotypic and functional characterization of T helper 17 (Th17) cells in patients with oral squamous cell carcinoma** (**PhD thesis work)**

Duration: Aug 2010 –July 2015

Summary**:** Th17 cells have been identified as a third independent T cell subset implicated in various inflammatory and autoimmune disorders. Recently it has also gained prominence in cancer immunity however their activity yielded conflicting data. Therefore we explored the phenotypic and functional characteristics of Th17 cells and its relationship with other T cell subsets in OSCC patients and healthy individuals as controls.

Th17 cell subsets ($CD4^+IL17A^+$/ $CD8^+IL17A^+$) were enumerated in PBMCs using Flow cytometry. The percentages were compared with that of healthy controls. The various T cell subsets namely $CD4^+T$, $CD8^+T$ and $CD4^+CD25^+FOXP3^+$ (Tregs) were correlated with Th17 prevalence in oral cancer patients to predict its possible role in oral cancer. These studies were validated by ELISA at protein levels. The serum levels of IL17A, TGFβ1, Th1 (IL2, IFNγ) and Th2 (IL4, IL10) were analyzed and found to be potential diagnostic markers. These findings were further correlated with the clinic-pathological features of oral cancer. The criss-cross relation of Th17-Treg cells was found to be a significant prognostic marker in the development of oral cancer. Th17 cells were then characterized in terms of its various activation (HLA-DR, CD25, CD69), homing (CD62L, CCR7, CCR6, CXCR4), effector (CD28, FOXP3, CD161) markers using five-color-flow cytometry. The cytokine profiling revealed plasticity of these cells with Th1 cells establishing three profound subsets based on the cytokines secreted namely Th17/1 ($IL17A^+IFN\gamma^+$), Th17/2 ($IL17A^+IL4^+$) and pro-inflammatory Th17 ($IL17A^+IL8^+$).

We then explored the functional role of Th17 cells *in vitro* on oral cancer cell lines (SCC-4, SCC-9 and SCC-25). The Th17 cells were sorted by magnetic cell sorting (MACs) and expanded by cell culturing in Th17 activating conditions. These were then co-cultured with oral cancer cell lines. The cytotoxicity assay (MTT reduction), apoptosis (Annexin-V binding assay) and expression of VEGF (by ELISA) in culture supernatant were done to find out the possible role of Th17

in oral cancer pathogenesis. Th17 cells but not rhIL17A cytokine directly were found to have anti-tumor activities *in vitro*.

This study helped in understanding the inter-relationship of Th17 cells with other immune cells in the immunopathology of oral cancer and thereby its contextual functions. Thus it may provide translational significance in developing more effective strategy for adoptive immunotherapy and novel treatment modalities of these tumors.

Post-Doc Resaerch Experience

Worked as SRF in AYUSH funded project "Investigation of anti-cancer activities of some select medicinal plants and their molecular targets on oral cancer cell lines" in the department of Biotechnology, AIIMS for 1 year (Nov 2015-Aug 2016)

Teaching Experience (3 yrs)

Working as Assistant Professor (Biochemistry) at PDM University, Bahadurgarh, Haryana. (Aug 2016- Present)

Research publications

1. **Gaur P**, Mittal M, Mohanti BK, Das SN. Functional variants of IL4 and IL6 genes and risk of tobacco-related oral carcinoma in high-risk Asian Indians. ***Oral Dis.*** 2011; 17(7): 720-6. **IF: 2**
2. **Gaur P**, Mittal M, Mohanti BK, Das SN. Functional genetic variants of TGF-β1 and risk of tobacco-related oral carcinoma in high-risk Asian Indians. ***Oral Oncol***. 2011; 47(12): 1117-21. **IF: 2.86**
3. **Gaur P,** Qadir GA, Upadhyay S, Singh AK, Shukla NK, Das SN. Skewed immunological balance between Th17 (CD4(+)IL17A (+)) and Treg (CD4 (+)CD25 (+)FOXP3 (+)) cells in human oral squamous cell carcinoma. ***Cell Oncol (Dordr)***. 2012; 35: 335-43. **IF: 3.56**
4. Jha R, **Gaur P**, Sharma SC, Das SN. Single nucleotide polymorphism in *hMLH1* promoter and risk of tobacco-related oral carcinoma in high-risk Asian Indians. ***Gene*** 2013; 526: 223-7. **IF: 2.08**
5. **Gaur P,** Singh AK, Shukla NK, Das SN. Inter-relation of Th1, Th2, Th17 and Treg cytokines in oral cancer patients and their clinical significance. ***Hum Immunol*** 2014; 75: 330-7. **IF: 2.14**

6. Gaur P, Shukla NK, Das SN. Phenotypic and functional dynamics of Th17 cells in oral squamous cell carcinoma and its clinical significance (abstract). ***J Carcinogenesis*** (eIssue International conference) 2012;11:S38.

7. Singh AK, **Gaur P**, Das SN. Natural killer T cell anergy, co-stimulatory molecules and immunotherapeutic interventions. ***Human Immunol*** 2014; 75: 250-60. **IF: 2.14**

8. Singh AK, **Gaur P**, Das SN. Differential dendritic cell-mediated activation and functions of invariant NKT-cell subsets in oral cancer. ***Oral Dis***. 2015; 21: e105-13. **IF:2**

9. Bharti V, **Gaur P**, Das SN. Molecular targets of *Cinnamomum zeylanicum* and its bio-active compound Cinnamaldehyde for anti-tumor activities against oral cancer. ***J Pharmacy res*** 2016; 10:493-501**. IF: 2.89**

10. Gaur P, Shukla NK, das SN. Phenotypic and functional characteristics of Th17 ($CD4^+IL17A^+$) cells in human oral squamous cell carcinoma and its clinicall relevance. Immunol. Invest. 2017:46:689-702. **IF:2.0**

11. Arora R, Bharti V**, Gaur P**, Das SN. Operculina turpethum extract inhibits growth and proliferation by inhibiting NF-κB, COX-2 and cyclin D1 and induces apoptosis by up regulating P53 in oral cancer cells. Archives of Oral Biol. 2017: 80:1-9. **IF: 2.0**

Book Chapter

1. Role of biotechnology in modern medicine. Das SN**, Gaur P**. Biotechnology in medicine and herbal drug development, Dr. Parveen Bansal & Dr. S.N. Das; Gulab Publisher: 1st edi. 2014. ISBN:978-81-92064-0-4.
2. Critical View of traditional medicine based clinical research. Das SN, **Gaur P**. Potentials and bottlenecks in clinical trials of herbal drugs, Dr. Parveen Bansal & Dr. S. E. Reddy; Gulab Publisher: 1st edi. 2015. ISBN:978-81-920643-9-0.
3. Role of biotechnology and bioinformatics in drug discovery. Das SN, **Gaur P**. Life sciences in medicine. Dr. Parveen Bansal & Dr. Ravinder Garg; Adbi Parwaaz Parkashan: 1st edi. 2016. ISBN: 978-93-85404-33-7

Technical Skills

Molecular Biology

Isolation of Genomic DNA, Plasmid & RNA, Agarose Gel Electrophoresis, Polymerase chain reaction, RT-PCR, Competent cell preparation, transformation and transduction experiments

Proteomics

Protein Expression and Purification, SDS-PAGE, Western blotting, colloidal coomassie blue staining,

Immunology

ELISA, Immuno Flourescence, Outcher-Lony Double Diffusion, FACS, Hybridoma

Computer Knowledge

Microsoft Office, NCBI tools, Primer designing, statistical software

Conferences/Workshops

- Attended National symposium ‘’New frontiers in cell Biology’’ organized by dept. of Biochemistry 2006, Deshbandhu College, DU, New Delhi, India..
- Attended National symposium ‘’Emerging trends in Biotechnology’’ organized by dept. of Biochemistry, 2007 Deshbandhu College, DU, New Delhi, India,
- Attended National symposium ‘’Translational research in health Sciences’’ organized by Society of Young Scientists, 2009 A.I.I.M.S, New Delhi, India.
- Attended Brainstorming session on “Translational Medicine and strengthening Biomedical Research in medical school system in India” held at Dr.Ramalingaswamy Board Room, 2010 A.I.I.M.S, New Delhi, India.
- Attended International conference ‘’New horizon in cancer research: Biology to prevention to therapy conference’’ organized by AACR (American Association for Cancer Research), 2011 held at Gurgaon, New Delhi, India.
- Attended National symposium ‘New horizon in basic and clinical research’’ organized by Society of Young Scientists, 2012 A.I.I.M.S, New Delhi, India.
- Attended Workshop on personal flow cytometry on ‘’BD Accuri C6 Personal Flow Cytometry’’ organized by BD Biosciences, 2012, BD FACS Academy, New Delhi, India.
- Attended 3rd International conference ‘’Frontiers in Carcinogenesis & Preventive oncology molecular mechanisms to therapeutics’’ organized by Carcinogenesis, 2012 at Dr. RML Hospital, New Delhi, India.

- ***Abstract published as proceedings of conference. Gaur P, Shukla NK, Das SN. Phenotypic and functional dynamics of Th17 cells in oral squamous cell carcinoma and its clinical significance. J Carcinogenesis (eIssue International conference) 2012;11:S38.***
- **Presented poster** ''Phenotypic and functional dynamics of Th17 cells in oral squamous cell carcinoma and its clinical significance'' at 3rd International conference ''Frontiers in Carcinogenesis & Preventive oncology molecular mechanisms to therapeutics'' organized by Carcinogenesis, 2012 held at Dr. RML Hospital, New Delhi, India.
- **Presented poster** "Inverse relationship between Th17 ($CD4^+IL17A^+$) and Treg ($CD4^+CD25^+FOXP3^+$) cells in human oral squamous cell carcinoma" at National symposium 'Recent trends in biological sciences' organized by Society of Young Scientists, 2014 A.I.I.M.S, New Delhi, India.
- **Presented poster** "Deregulated balance and clinical relevance of Th1, Th2, Th17 and Treg cytokines in oral cancer patients" at 12th International conference of the Asian Clinical oncology society and 35th Annual convention of Indian Association for cancer research mid-term conference IASO, Hotel The Ashok, New Delhi, India.
- ***Abstract published as proceedings of conference. Gaur P, Shukla NK, Das SN. The potential anti-tumor activities of Th17 cells on oral squamous carcinoma cells in vitro. J Carcinogenesis (eIssue International conference) 2016.***

Talks Presented at AIIMS

- RNA Interference
- Spliceosomes meet Telomerase
- An Immunomodulatory molecule of symbiotic bacteria directs host immune system maturation

Personal Details

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Status: Married

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Date:

Place: New Delhi (Dr. Poonam Gaur)

References

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ORAL DISEASES

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ORIGINAL ARTICLE

Differential dendritic cell-mediated activation and functions of invariant NKT-cell subsets in oral cancer

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OBJECTIVES: Invariant natural killer T (iNKT) cells are unique subset of glycolipid-reactive T lymphocytes with potent antitumour characteristics. This study was planned to understand Th-like cytokine profiles of iNKT-cell subsets and modulation of their functions in response to glycolipid ligand and tumour cell lysate (TL).

SUBJECTS AND METHODS: Cytokine profile of iNKT-cell subsets was evaluated from the peripheral blood of eight oral squamous cell carcinoma (OSCC) patients by flow cytometry and enzyme-linked immunosorbent assay (ELISA), while antitumour activity of iNKT cells was measured by methyl tetrazolium salt assay.

RESULTS: $CD4^+$ ($CD4^+CD8^-$) iNKT subset from OSCC patients showed significant ($P < 0.01$) expansion and higher IL-4 production following activation with α-GalCer-pulsed DCs, while $CD4^-CD8^-$ double negative (DN) and $CD8^+$ ($CD4^-CD8^+$) iNKT subsets produced IFN-γ predominantly. iNKT cells showed significantly ($P = 0.02$) increased secretion of IFN-γ and enhanced cytotoxicity to KB and SCC-4 tumour cells in response to α-GalCer and TL-pulsed DCs.

CONCLUSION: It appears that mutual balance/ratio of iNKT subsets may be important for their effector functions. Selectively expanded DN and $CD8^+$ iNKT cells with α-GalCer and TL may be a better candidate vaccine for iNKT-cell-based adoptive cancer immunotherapy.

Oral Diseases (2015) 21, e105–e113

Keywords: iNKT subsets; α-galactosylceramide; cytokine; dendritic cells; cytotoxicity; oral cancer

Introduction

Natural killer T (NKT) cells are a small population of thymus-derived T cells that express αβ T-cell receptor (TCR) and natural killer (NK) cell lineage markers and possess functional properties of both conventional T and NK cells (Godfrey *et al*, 2010; Berzins *et al*, 2011). Type I NKT cells, often referred to as invariant (i) NKT cells, express an invariant T-cell receptor (TCR) α chain (Vα24-Jα18 in humans and the homologous Vα14-Jα18 in mice) with a conserved CDR3 region that is paired with semi-invariant TCR-β chain (Vβ11 in humans and Vβ8, Vβ7 or Vβ8.2 in mice). iNKT cells are autoreactive for the non-classical major histocompatibility complex (MHC) class I-like molecule CD1d (Bendelac *et al*, 1995) and have a strong response to the glycosphingolipid antigens such as the marine sponge-derived agent α-galactosylceramide (α-GalCer; KRN-7000) presented by CD1d (Kawano *et al*, 1997; Burdin *et al*, 1998).

The most remarkable property of iNKT cells is their capacity to produce substantial amounts of cytokines, such as IL-4, IFN-γ and IL-12 upon TCR engagement (Godfrey and Kronenberg, 2004). Activated iNKT cells in turn trigger a cascade of events by activating a variety of cells such as T cells, B cells, NK cells and macrophages and recruit myeloid dendritic cells (DCs) (Camaud *et al*, 1999; Metelitsa *et al*, 2001; Kronenberg and Gapin, 2002). Therefore, iNKT cells are considered as a link between innate and adaptive immunity (Van Kaer *et al*, 2011). These cells function as an adjuvant against tumours by activating other antitumour cytolytic cells through release of Th1 cytokines (Motohashi and Nakayama, 2008). In this manner, activated iNKT cells acquire potent immunoregulatory properties which have been successfully explored for regulating autoimmune disorders or promoting tumour rejection (Jahng *et al*, 2001; Terabe and Berzofsky, 2008) besides development of vaccine adjuvants (Kim *et al*, 2008) and designing various therapeutic clinical trials (reviewed in ref. (Singh *et al*, 2014)). Reportedly, iNKT cells are compromised in number and functions in patients with cancer (Tahir *et al*, 2001; Motohashi *et al*, 2002; Dhodapkar *et al*, 2003; Crough *et al*, 2004; Molling *et al*, 2005). Therefore, restoration of iNKT-cell numbers as well as its functions, especially in terms of IFN-γ production, has been the primary aim of therapeutic approaches such as direct immunization or adoptive transfer of in vitro-activated iNKT cells (Singh

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Functional genetic variants of *TGF-β1* and risk of tobacco-related oral carcinoma in high-risk Asian Indians

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SUMMARY

Transforming growth factor (TGF)-β1, the most abundant isoform of TGF-β have been implicated in various stages of carcinogenesis such as epithelial to mesenchymal transition, enhanced expression of metalloproteases, down-regulation of cellular adhesion molecule, increased tumor motility and angiogenesis as well as local and systemic immunosuppression leading to a more aggressive and metastatic behavior. We assessed the association of TGF-β1 functional genetic polymorphisms at codon 10 (869 T > C) and 25 (915 G > C) of exon 1 in 140 patients with tobacco-related oral squamous cell carcinoma (OSCC) and 120 normal subjects by PCR–RFLP. The frequency of 869 CC genotype and C allele were significantly higher in patients as compared to controls (P_c 0.024 and 0.0004, respectively) while no significant difference was observed in the frequency of 915 CC genotype and C allele. In logistic regression analysis CC genotype (OR 3.87; 95% CI, 1.70–8.81) and C allele (OR, 2.28; 95% CI, 1.51–3.50) appeared as susceptible while TT genotype and T allele as protective. In addition C_{869}–C_{915} haplotype with OR of 2.48 at 95% CI, 1.51–4.05 significantly (P = 0.0005) increased the risk of tobacco-related OSCC in Asian Indians.

Introduction

Oral cancer is the sixth most common cancer worldwide and a major health problem in India. It ranks number one among men and number three among women in India.[1] Interleukins and cytokines play an important role in the pathogenesis of many solid cancers. A variety of cytokines and growth factors produced by tumor cells and tumor infiltrating lymphocytes appear to regulate tumor cell growth, progression, angiogenesis and metastases.[2] Single nucleotide polymorphisms (SNPs) in cytokine genes may influence the expression[3] or function of cytokines via cytokine network and many have been evaluated for their role in inflammatory diseases and cancer predisposition.[4,5] Earlier we have reported deregulated expression of Th2 cytokines in patients with tobacco related oral squamous cell carcinoma (OSCC).[6,7]

TGF-β is a pleiotropic cytokine that has important role in various cellular processes such as growth, proliferation, differentiation, apoptosis, angiogenesis and formation of extracellular matrix.[8] All three isoforms (TGF-β1, β2, and β3) of TGF-β has a tissue specific expression and each is encoded by a distinct gene. TGF-β1 represents the most abundant form expressed in endothelial cells, connective tissue and haematopoietic cells.[9] TGF-β1 plays dual roles in tumor growth. In the early stage of tumorigenesis it acts as a tumor inhibitor by suppressing epithelial cell proliferation, but in the advanced stages it acts as tumor promoter. At late stages TGF-β1 can promotes epithelial to mesenchymal transition, enhances expression of metalloproteases, down regulates cellular adhesion molecule, increases tumor motility, angiogenesis and causes local and systemic immunosuppression resulting into a more aggressive and metastatic behavior of tumor.[10–12]

TGF-β1 gene is located on chromosome 19q13. Out of several polymorphisms of *TGF-β1*, two are located on codon 10 (869 T > C::Leu/Pro) and codon 25 (915 G > C::Arg/Pro) of exon 1 that encodes leader sequence of the protein and have functional importance in modulating its transmembrane transport.[13] Several studies have been conducted to identify role of codon 10 T > C polymorphism of *TGF-β1* in cancer development and progression but results are inconsistent and inconclusive. Codon 10 C allele was found to be related to increased susceptibility to nasopharyngeal[14] and esophageal squamous cell carcinoma[15] in Chinese population, however in Japanese T allele at codon 10 was associated with increased susceptibility to prostate cancer[16] while in Caucasians and African Americans it did not show any significant association.[17] Thus there seems to be ethnic variation in the association of *TGF-β1* gene SNPs and genetic susceptibility to different cancers. To the best of our knowledge, no reports are available on *TGF-β1* gene polymorphisms and its association with the risk of tobacco related OSCC in Asian Indians.

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ORIGINAL PAPER

Skewed immunological balance between Th17 ($CD4^+IL17A^+$) and Treg ($CD4^+CD25^+FOXP3^+$) cells in human oral squamous cell carcinoma

Poonam Gaur · Gulam Abdul Qadir · Shilpy Upadhyay · Avadhesh Kumar Singh · Nootan Kumar Shukla · Satya Narayan Das

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Abstract

Background Several studies have documented modulation of Th17 and T regulatory (Treg) cells in various human malignancies which may vary with the type and extent of the disease. However, such data in patients with oral cancer is scarce and hence the current study was designed to elaborate the immunological balance between these two T cell subsets in oral cancer.

Methods and results We analyzed various T cell subsets in the peripheral blood of 45 oral squamous cell carcinoma (OSCC) patients and 40 healthy volunteers. We found that, compared with the healthy controls, patients had a significantly ($p<0.0001$) higher proportion of both Th17 ($CD4^+IL17A^+$) and Treg ($CD4^+CD25^+FOXP3^+$) cells, which further showed a reciprocal balance in relation to clinico-pathological parameters in patients. We also detected a circulating $CD8^+$ subset of these cells in both patients and healthy controls, although the difference between the two groups was statistically insignificant. Higher frequencies of Th17 cells were found in patients with early stages and without lymph node involvement, while an increased prevalence of Tregs was associated with higher clinical stages and lymph node involvement. Moreover, Th17 cells were quantitatively and positively correlated to $CD4^+T$ and $CD8^+T$ cells and inversely correlated with Tregs. Contrarily, Tregs showed a negative association with $CD4^+T$ and $CD8^+T$ cells.

Conclusions Our results suggest an increase in Th17/Treg ratio in early stages and a decrease in this ratio in higher stages of oral cancer. Such counter regulation of Th17 and Tregs may be a significant prognostic factor in oral cancer patients.

Keywords T cell subsets · Th17 · Tregs · Oral cancer

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Surgical Oncology,
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1 Introduction

Oral squamous cell carcinoma (OSCC) accounts for up to 7.4 % of total malignancies in India with an age-standardized incidence rate of 7.5 per 100,000 [1]. Such a high incidence rate is posing formidable challenges to oncologists. Apart from various factors including tobacco, alcohol, viral infection and physical irritation, the host immune system also plays a pivotal role in oral carcinogenesis [2]. Induction of immune surveillance is often observed in the early stage. However later on the local and systemic cell mediated response is suppressed in cancer patients leading to the escape of tumor cells [3]. Oral cancer cells may escape the attack of the immune response by having an excess of immunosuppressive factors like PGE_2, TGF-β, IL10 and Fas ligand in their vicinity that may reduce total immuno-competent cell counts [4]. This detrimental immune-tolerant capacity is supposed to be induced by $CD4^+CD25^+FOXP3^+$ T regulatory

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Inter-relation of Th1, Th2, Th17 and Treg cytokines in oral cancer patients and their clinical significance

Poonam Gaur [a], Avadhesh Kumar Singh [a], Nootan K. Shukla [b], Satya N. Das [a,*]

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ABSTRACT

Background: Altered cytokine production can lead to immune dysfunction in cancer patients. Hence, we investigated the cytokine balance in oral squamous cell carcinoma (OSCC) patients and their significance in providing new therapeutic insights.
Methods: We quantified Th17 (IL17A), Treg (TGFβ1), Th1 (IL2, IFNγ) and Th2 (IL4, IL10) like cytokines in the sera of 78 cases and 39 controls by ELISA. The intracellular expression of these cytokines was analyzed in 10 subjects from each group by flow cytometry.
Results: Serum levels of IL17A, TGFβ1, IL4 and IL10 were significantly higher while IL2 and IFNγ were relatively lower in patients as compared to controls. TGFβ1 ($r = 0.55$), IL4 ($r = 0.75$) and IL10 ($r = 0.80$) significantly ($P = 0.0001$) correlated with disease progression and their elevated levels showed increased odd ratios of approximately 18, 14 and 37, respectively. IL17A appeared as a risk factor (OR = 2.21, 95% CI = 0.86–5.42) although statistically insignificant. The levels neither correlated with disease progression nor with TGFβ1, IL4 and IL10 but showed positive association with IL2 ($r = 0.61$, $P = 0.0001$) and IFNγ ($r = 0.24$). Flow cytometry data also showed similar trend.
Conclusions: We reported a dominant TGFβ1 and Th2 (IL4, IL10) polarization with a borderline elevation of IL17A while, a suppression of Th1 (IL2, IFNγ) cytokines in OSCC patients.

1. Introduction

Oral cancer is a major life threatening disease worldwide particularly in India. According to a recent report approximately 2.6×10^5 new cases and 1.2×10^5 number of deaths had been attributed to this devastating disease in the year 2008 globally [1]. It is tremendously increasing incidence rate and such high mortality owing to late diagnosis is posing formidable challenge to oncologists to search for a new better diagnostic and treatment approaches exploiting immunology of oral cancer. Exploring the intricate balance of cytokines in oral cancer patients may provide insights to these questions.

Cytokines are immuno-modulatory proteins whose aberrant expression leads to deregulation of immune response, excessive cell growth, malignant transformation, tumor cell escape from surveillance mechanism and metastasis [2]. The diagnostic potential and prognostic relevance of various cytokines, growth and angiogenetic factors have been well documented in different type of cancers over time [3–7] but there has been paucity of such serological biomarkers in case with oral cancer [8].

There are four main subsets of $CD4^+$ T helper (Th) cells based on their distinct cytokine repertoire that governs the overall immune response through delicate network. These are Th1, Th2, Th17 and T regulatory (Tregs) cells. Th1 cells produce hallmark IL2, IL12, IFNγ and TNFα cytokines involved in cell-mediated pro-inflammatory response, these exert potent anti-tumor effects by activating $CD8^+$ cytolytic T lymphocytes (CTLs), NK mediated cytotoxicity and up-regulation of MHC expression on APCs. Conversely, Th2 cells secrete IL4, IL5, IL6, IL10 and IL13 which mediate anti-inflammatory

Abbreviations: OSCC, oral squamous cell carcinoma; Th, T helper; IL, interleukin; Treg, T regulatory; TGFβ1, transforming growth factor-beta1; IFNγ, interferon-gamma; TNFα, tumor necrosis factor-alpha; CTLs, cytolytic T lymphocytes; NK, natural killer cells; MHC, major histocompatibility complex; APCs, antigen presenting cells; ROS, reactive oxygen species; VEGF, vascular endothelial growth factor; LOD, lower limit of detection; PBMCs, peripheral blood mononuclear cells; ROC, receiver operating characteristics curve.

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ORIGINAL ARTICLE

Functional variants of *IL4* and *IL6* genes and risk of tobacco-related oral carcinoma in high-risk Asian Indians

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BACKGROUND: Tobacco-related oral squamous cell carcinoma (OSCC) is one of the most common cancers involving Indian males. We assessed the association of *IL4* promoter –589 T>C, –33 T>C, and *IL6* –174 G>C functional genetic polymorphisms with tobacco-related OSCC in Asian Indians.

PATIENTS AND METHODS: The *IL4* and *IL6* promoter polymorphisms were assessed in 140 patients with OSCC and 120 normal subjects by PCR–RFLP technique, and significance of the data was determined using chi-square test.

RESULTS: The frequency of TC, CC genotype, and C allele at *IL4* promoter sites –589 and –33 were higher in patients when compared with controls. Consequently, TC/CC genotypes and C allele at both sites appeared as susceptible. However, *IL6* –174 G>C single-nucleotide polymorphisms (SNP) appeared to be protective in patients with OSCC. Of eight haplotypes, five were associated with two- to seven-fold increased risk of tobacco-related OSCC. These SNPs further showed heterogeneity among different ethnic population, but their distribution in Asian Indians stand closer to other Asian populations.

CONCLUSIONS: In this study, *IL4* –589 CC, –33 CC genotype, and *C allele at both sites appeared to be susceptible, while *IL6* –174 CC genotype and *C allele appeared to be protective in patients with OSCC; hence, these SNPs may be a potential prognostic markers for tobacco-related OSCC in Asian Indians.
Oral Diseases (2011) 17, 720–726

Keywords: IL4; IL6; single-nucleotide polymorphisms; haplotype; oral cancer; Asian Indians

Introduction

Oral cancer is the largest category of head and neck cancer. Worldwide an estimated 400 000 new cases of oral cancer are diagnosed every year with two-thirds of the cases occurring in developing countries like Sri Lanka, India, Pakistan, and Bangladesh (GLOBOCAN, 2005). Although external carcinogens and poor lifestyle habits such as tobacco, alcohol consumption and low-grade diet play a pivotal role in oral cancer development, strong genetic predisposition may further contribute to the susceptibility to the disease. Cytokines have been considered to play an important role in carcinogenesis. They may either be involved in the anti-tumor effector immune mechanisms or may enhance malignant transformation and tumor growth. They are produced by both host stromal and immune cells as well as by the cancer cells in the same microenvironment. But their relative concentration, receptor expression patterns, etc. decide the direction of their action. Broadly, Th1 type cytokines like IFN-γ, IL2, and IL12 are required for anti-tumor immunity, whereas Th2 type and several inflammatory cytokines like CSF-1, IL1 family, TNF, and TGF β favor tumor development (Smyth *et al*, 2004). We have earlier reported deregulated expression of Th2 cytokines in tobacco-related oral squamous cell carcinoma (Agarwal *et al*, 2003).

IL4, a 20-kDa glycoprotein, is a member of four α-helical cytokine family. It is produced by activated $CD4^+$ T cells, mast cells, and basophils. It is an autocrine growth factor for differentiation and expansion of Th2 subset, responsible for B cell switching to IgE production, antagonizes IFN-γ function, inhibits macrophage activation and reportedly shows anti-tumor activity on different cancer cells such as colon, breast (Toi *et al*, 1992), and renal carcinoma (Golumbek *et al*, 1991; Yu *et al*, 2004). However, we have earlier shown an up-regulated expression of IL4 in patients with tobacco-related oral squamous cell carcinoma (OSCC) (Agarwal *et al*, 2003; Manchanda *et al*, 2006).

Interleukin 6 (IL6), another cytokine of Th2 type, is a 25-kDa-long glycoprotein having multifunctional effect on various physiological and pathophysiological processes like inflammation, bone metabolism, synthesis of CRP, and carcinogenesis (Diehl and Rincon, 2002). It is synthesized by phagocytes, vascular endothelial cells, and fibroblasts. It has been demonstrated that IL6 acts as a potent stimulator of cancer metastasis by

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Chapter 1
Role of Biotechnology and Bioinformatics in Drug Discovery

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Introduction

Drug discovery and development is a complicated, high risk and resource consuming process but at the same time it is highly rewarding. It requires involvement of technological expertise, huge capital investment apart from ethical and manufacturing regulations before any drug is being put to use to a common man. The two branches that have positive impact on pharmaceutical industry reducing the cost and risk factors are Bioinformatics and Biotechnology. While Bioinformatics deals with the screening of suitable targets involved in a disease and then searching/computing another lead compound that can mimic/alter the action of the target compound *in silico*. Biotechnology, on the other hand has enabled large scale production of bio-pharmaceuticals such as peptides, proteins, enzymes, hormones, monoclonal antibodies, cytokines, antisense drugs and so on that have eased the burden on traditional methods of chemical synthesis of drugs. In the current chapter we will highlight the roles of these two crucial branches in modern drug discovery.

Bioinformatics as a Powerful Tool for Drug Designing

Bioinformatics is the study of analysis of biological data using computer programming, mathematics and statistics. The most important achievement of bioinformatics is the human genome project which was mapped in 2001. In the recent years, bioinformatics has eased the burden of generating more efficient and specific drugs in a short period of time and involving minimal risk. It has made easy for the researchers to target the molecules in the *in vitro* environment giving very efficient results. In fact now a separate branch of translational medicine known as computer aided drug design (CADD) has been developed that deals with the preliminary target validation cutting the experimental costs tremendously (1-2).

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Chapter 2

Role of Biotechnology in Modern Medicine

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INTRODUCTION

Biotechnology, an emerging key technology of 21st century that has started as commercial use of micro-organisms merely in brewing sector has now opened up new avenues of refashioning life on earth. With several decades of sincere efforts of scientific intellect and the advent of whole plethora of techniques of modernization, it had made its roots deep into various sectors viz. food, fodder, agriculture, marine technology, chemical industry, forensics, bio-terror, diagnostics, medicine and pharmaceuticals. The most fruitful impact of biotechnology is undoubtedly in health care sector that has stupendously improved the human health and life expectancy. About 20% of commercialized medicines, 50% of under trial and 40% of anti-cancer drugs are the gifts from medical biotechnology to mankind. The various products like recombinant vaccines, DNA vaccines, antibiotics, antibodies, blood clotting factors, cytokines, hormones, enzymes have ease the burden on health care personnel. In addition, tissue engineering that exploits the endowed power of pluripotent stem cells aims at functional regeneration of tissues through culturing is also a pivotal branch of biotechnology. It had excited the common man for the first time with the birth of 'Dolly' from the nuclei of an udder cell. The approach of reprogramming egg cell cytoplasm has revolutionized the concept of 'animal bioreactors'. Genetically engineered embryo to produce cattle with desired protein pharmaceuticals in milk, meat, insect resistant wool from sheep will prove a milestone in this era. The rapid development in this field have made it possible to detect abnormalities in the genome of unborn babies and to correct it with one time gene therapy at stem cell level, making the dream of designer babies come true. While, this has led to serious ethical debates on human cloning, specific organ cloning and transplantation is considered as appropriate and eventually feasible.

Modern biotechnology has become an ideal ground for pharma companies to flourish. Drug discovery is a very rapidly evolving field

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Chapter 1

CRITICAL VIEW OF TRADITIONAL MEDICINE BASED CLINICAL RESEARCH

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Introduction

Traditional medicines is a dynamic term coined for a diverse health practices based on theories, beliefs and experiences of different cultures and times, incorporating plants, animals, and/or mineral based medicines, spiritual therapies, manual techniques, exercises for the maintenance of health, as well as in prevention, diagnosis, improvement and treatment of illnesses. Since the time immemorial herbal medicines have made a great contribution in traditional medical practice in different civilizations. World Health Organization (WHO) estimates that 80% of the world's population currently relies on herbal medicines for major healthcare while 25% of all modern medicines are directly or indirectly derived from plant source.

Emphasizing their importance, a comprehensive programme for the identification, cultivation, preparation, evaluation and conservation of herbal medicines has been developed by WHO to endorse their safe and effective use. In recent times a great boon has been given to clinical research in this field. The goal of these studies is not only to evaluate safety and efficacy of herbal medicines but also to promote their rational use. However, there are several contrasting pros and cons of clinical research in traditional medicine. This chapter highlights the key advantages and disadvantages of clinical research on traditional medicine as well as suggests some guidelines for the standardized research on these herbal medicines.

Advantages of traditional medicine- based clinical research

In the recent past natural therapies have gained popularity not only in developing countries but it has stretched its limits to the developed countries as well. Such a dramatic public interest in traditional medicine owes to its several contributing advantages:

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Single nucleotide polymorphism in *hMLH1* promoter and risk of tobacco-related oral carcinoma in high-risk Asian Indians

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ABSTRACT

hMLH1 is a member of mismatch repair genes (MMR) that plays a crucial role in correcting replication errors, cell cycle arrest, apoptosis and oxidative stress. We explored the risk associated with *hMLH1* −93 A>G (rs 1800734) single nucleotide polymorphism (SNP) with the oral squamous cell carcinoma (OSCC) in Asian Indians. We genotyped 242 patients with tobacco-related OSCC and 295 healthy controls by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The frequency of AA genotype was found to be significantly ($P_c = 0.0006$) lower in patients as compared to the controls (21.48% vs. 47.8%) while GG genotype showed significantly higher ($P_c < 0.0006$) prevalence in patients as compared to the healthy controls (41.32% vs. 13.06%). In logistic regression analysis AG (adjusted OR = 1.95, 95% CI = 0.72–5.26) and GG genotype (adjusted OR = 4.5, 95% CI = 1.54–13.16, $P = 0.006$) appeared susceptible when compared with the wild-type AA genotype. The allelic distribution showed that variant C allele is significantly higher ($P_c < 0.0004$) in patients and associated with increased risk (adjusted OR = 2.36, 95% CI = 1.33–4.19, $P = 0.003$) as compared to the wild-type A allele. Altogether, our results suggest that the *hMLH1* −93 A>G polymorphism is associated with the higher risk of tobacco-related OSCC in Asian Indians and could be useful in screening population at a higher risk.

1. Introduction

Oral cancer is the sixth most common cancer worldwide with a global annual incidence rate of over 640 000, of which 62% arise in developing countries including India and Southeast Asia (Ferlay et al., 2010). The primary etiological factors include usage of tobacco/areca nut/betel leaf, marijuana, human papilloma virus, Epstein–Barr virus infections, alcohol, poor diet, exposure to radiation and genetic predisposition (Sturgis et al., 2004).

Oral squamous cell carcinoma (OSCC) is preceded by benign lesions such as leukoplakia and submucous fibrosis which are specifically caused by N-nitrosamines (TSNAs)/benzo-pyrene like classical carcinogens found in tobacco (Hecht, 2003; Tang et al., 1992). These carcinogens produce pyridyl hydroxybutyl/benzo(a)pyrene diol epoxide (BPDE) adducts in DNA and induce frequent mutations, DNA damage and genomic-wide instability. These events subsequently lead to activation of oncogenes, inactivation of tumor suppressor genes and ultimately cancer development (Barnes, 2002). The pivotal set of genes in this regard is the DNA repair system that plays crucial role in maintaining genomic integrity during DNA replication, correcting post-replicative errors, random mutations, oxidative stress and the aging process (Dixon and Kopras, 2004). There are 5 major DNA repair pathways in humans namely nucleotide excision repair, base excision repair, mismatch repair, and homologous recombinational and non homologous end joining which together involve approximately 70 genes (Bernstein et al., 2002). Any genetic or epigenetic alteration in these genes may have a serious implication in one's DNA repair capacity (DRC) (Qiao et al., 2002), which in turn changes the susceptibility to cancer development (Goode et al., 2002).

hMLH1 is a key component of the mismatch repair system that plays crucial role in recognition of nucleotide mismatch and together with MSH2 recruits whole repair machinery to the error site (Ilyas et al., 1999). Besides, it is important for other cellular processes such as cell cycle arrest, oxidative stress and apoptosis (Jiricny, 2006). A number of studies have explored the association of its profound SNPs (Wehner et al., 1997) with the susceptibility of developing various human malignancies including lung (Lo et al., 2011; Park et al., 2004), breast (Lee et al., 2005), ovarian (Harley et al., 2006), endometrial (Reiner et al., 2006), colorectal (Raptis et al., 2007) and hereditary non-polyposis colorectal cancer syndrome (HNPCC) (Mitchell et al., 2002). Out of all, *hMLH1* −93 A>C has been studied extensively because of its prevalence and functional significance. hMLH1 gene is composed of 19 exons spanning

Abbreviations: hMLH1, human MutL homolog 1; MMR, mismatch repair; SNP, single nucleotide polymorphism; OSCC, oral squamous cell carcinoma; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; BPDE, benzo(a)pyrene diol epoxide; DRC, DNA repair capacity; MSH2, MutS homolog 2; HNPCC, hereditary non-polyposis colorectal cancer syndrome; GT-IIB, GT-insert 2B; NF-IL6, interleukin 6-regulatory nuclear factor; UICC, Union for International Cancer Control.

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ORIGINAL ARTICLE

Differential dendritic cell-mediated activation and functions of invariant NKT-cell subsets in oral cancer

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OBJECTIVES: Invariant natural killer T (iNKT) cells are unique subset of glycolipid-reactive T lymphocytes with potent antitumour characteristics. This study was planned to understand Th-like cytokine profiles of iNKT-cell subsets and modulation of their functions in response to glycolipid ligand and tumour cell lysate (TL).

SUBJECTS AND METHODS: Cytokine profile of iNKT-cell subsets was evaluated from the peripheral blood of eight oral squamous cell carcinoma (OSCC) patients by flow cytometry and enzyme-linked immunosorbent assay (ELISA), while antitumour activity of iNKT cells was measured by methyl tetrazolium salt assay.

RESULTS: $CD4^+$ ($CD4^+CD8^-$) iNKT subset from OSCC patients showed significant ($P < 0.01$) expansion and higher IL-4 production following activation with α-GalCer-pulsed DCs, while $CD4^-CD8^-$ double negative (DN) and $CD8^+$ ($CD4^-CD8^+$) iNKT subsets produced IFN-γ predominantly. iNKT cells showed significantly ($P = 0.02$) increased secretion of IFN-γ and enhanced cytotoxicity to KB and SCC-4 tumour cells in response to α-GalCer and TL-pulsed DCs.

CONCLUSION: It appears that mutual balance/ratio of iNKT subsets may be important for their effector functions. Selectively expanded DN and $CD8^+$ iNKT cells with α-GalCer and TL may be a better candidate vaccine for iNKT-cell-based adoptive cancer immunotherapy.

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Keywords: iNKT subsets; α-galactosylceramide; cytokine; dendritic cells; cytotoxicity; oral cancer

Introduction

Natural killer T (NKT) cells are a small population of thymus-derived T cells that express αβ T-cell receptor (TCR) and natural killer (NK) cell lineage markers and possess functional properties of both conventional T and NK cells (Godfrey *et al*, 2010; Berzins *et al*, 2011). Type I NKT cells, often referred to as invariant (i) NKT cells, express an invariant T-cell receptor (TCR) α chain (Vα24-Jα18 in humans and the homologous Vα14-Jα18 in mice) with a conserved CDR3 region that is paired with semi-invariant TCR-β chain (Vβ11 in humans and Vβ8, Vβ7 or Vβ8.2 in mice). iNKT cells are autoreactive for the non-classical major histocompatibility complex (MHC) class I-like molecule CD1d (Bendelac *et al*, 1995) and have a strong response to the glycosphingolipid antigens such as the marine sponge-derived agent α-galactosylceramide (α-GalCer; KRN-7000) presented by CD1d (Kawano *et al*, 1997; Burdin *et al*, 1998).

The most remarkable property of iNKT cells is their capacity to produce substantial amounts of cytokines, such as IL-4, IFN-γ and IL-12 upon TCR engagement (Godfrey and Kronenberg, 2004). Activated iNKT cells in turn trigger a cascade of events by activating a variety of cells such as T cells, B cells, NK cells and macrophages and recruit myeloid dendritic cells (DCs) (Camaud *et al*, 1999; Metelitsa *et al*, 2001; Kronenberg and Gapin, 2002). Therefore, iNKT cells are considered as a link between innate and adaptive immunity (Van Kaer *et al*, 2011). These cells function as an adjuvant against tumours by activating other antitumour cytolytic cells through release of Th1 cytokines (Motohashi and Nakayama, 2008). In this manner, activated iNKT cells acquire potent immunoregulatory properties which have been successfully explored for regulating autoimmune disorders or promoting tumour rejection (Jahng *et al*, 2001; Terabe and Berzofsky, 2008) besides development of vaccine adjuvants (Kim *et al*, 2008) and designing various therapeutic clinical trials (reviewed in ref. (Singh *et al*, 2014)). Reportedly, iNKT cells are compromised in number and functions in patients with cancer (Tahir *et al*, 2001; Motohashi *et al*, 2002; Dhodapkar *et al*, 2003; Crough *et al*, 2004; Molling *et al*, 2005). Therefore, restoration of iNKT-cell numbers as well as its functions, especially in terms of IFN-γ production, has been the primary aim of therapeutic approaches such as direct immunization or adoptive transfer of in vitro-activated iNKT cells (Singh

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