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Leading article

Association of Single Nucleotide Polymorphisms in Cell Cycle Regulatory Genes with Oral Cancer Susceptibility

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Abstract

Alterations in the regulation of the cell cycle are strongly linked to tumorigenesis, so genetic variants in genes critical to control of the cycle are good candidates to have their association with susceptibility to oral cancer assessed. In this hospital-based, case-control study of 445 patients who had been newly-diagnosed with oral cancer and 449 unaffected controls, we used a multigenic approach to examine the associations among a panel of 10 selected polymorphisms in the pathway of the cell cycle that were possibly susceptible to oral cancer. Six of 9 single nucleotide polymorphisms in the cell cycle showed significant risks for oral cancer, the highest risk being evident for p27 (rs34329; Odds ratio 3.05, 95% CI 2.12 to 4.40). A significant risk of oral cancer was also evident for individual polymorphisms of cyclin E (rs1406), cyclin H (rs3093816), cyclin D1-1 (rs647451), cyclin D2 (rs3217901) and Rb1-2 (rs3092904). The risk of oral cancer increased significantly as the number of unfavourable genotypes in the pathway increased, and so the results point to a stronger combined effect of polymorphisms in important cell cycle regulatory genes on predisposition to oral cancer.

Keywords: Cell cycle; Single nucleotide polymorphism; Oral cancer; Susceptibility

Introduction

Oral cancer is the sixth most common malignancy worldwide,[1] with a high incidence on the Indian subcontinent.[2] Because traditional clinical markers tend to be subjective, recent research has focused more on the development of new biological markers for early detection.[3] Progression of the mammalian cell cycle is controlled by a subfamily of cyclin-dependent kinases, the activity of which is modulated by several activators and inhibitors.[4] Studies of inherited variations in genes in the cell cycle have suggested that genotypes in this pathway may be associated with the risk of cancers in the breast,[5] prostate,[6] lung,[7] bladder,[8] ovary,[9] and oral cavity.[10,11]

However, the conventional approach using a single candidate gene has yielded inconsistent data across different studies, even for the same single nucleotide polymorphism. Recently a polygenic model of cancer was proposed that considers genetic susceptibility to cancer as a global mechanism, with susceptibility being defined by low risk alleles in multiple candidate genes in the same pathway.[12–14] Alterations in the expression of proteins in the cell cycle have been previously reported in both premalignant and malignant lesions that arise in the oral cavity.[15,16] A recent study has also shown

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that genetic polymorphisms in the control of pathway genes in the cell cycle may contribute to the risk of premalignant oral lesions.[17]

The aim of this case control analysis was therefore to use a pathway-based approach to assess the association among 10 single nucleotide polymorphisms from 8 cell cycle genes and the susceptibility to oral squamous cell carcinoma (SCC). Our data suggest that subjects with a larger number of unfavourable genetic variations in the genes that control the cell cycle are at an increased risk of oral cancer, which confirms the importance of taking a multigenic, pathway-based approach to genetic susceptibility.

Patients, material, and methods

Group studied and samples

We studied randomly-selected patients diagnosed with oral SCC at the Regional Cancer Centre, Trivandrum, India between 2006 and 2009. Patients with second primary tumours or with a previous history of treatment for cancer were excluded. A prestructured questionnaire was used to collect information on various personal, socioeconomic, and clinical variables, including their oral habits, which include chewing (betel quid with tobacco or tobacco alone), smoking, and alcohol consumption. They were recorded only if the patient had had the same habit for more than 2 years continuously. Ethics approval was obtained from Human Ethics Committee of the institute for the collection and research use of all samples of blood and tumour used in this study. All participants provided written informed consent in accordance with the guidelines of the Institutional Review Board. Follow-up data on all patients was obtained from medical records and when needed, from a telephone interview with the patient. For controls, we recruited subjects with no previous history of any type of cancer who attended the nearby polyclinic for minor ailments. Controls were matched with cases by age (within 5 years) and sex. However, because of technical problems we were unable to obtain data on oral habits from the control samples. All participants had 2 ml blood drawn into heparinised tubes and stored at -80 °C until DNA had been extracted. In a few cases where blood samples were not available, the DNA was extracted from tissue samples collected at the time that the investigative biopsy was taken before treatment.

Selection of genes and single nucleotide polymorphisms

The most critical phase in the cell cycle is the transition from G1 to S, which is concerned with surmounting the 'restriction point - R'.[4] In the present study we concentrated mainly on single nucleotide polymorphisms in genes involved in G1 to S transition, such as cyclin D1, cyclin D2, cyclin D3, cyclin E, cyclin H, p27, cdk5, and retinoblastoma. The criteria for selection were: an established intragenic location, more than 10% variant (minor) allele frequency in the tested population, and single mucleotide polymorphisms that had previously been evaluated for genetic susceptibility to cancer (Table 1).

Molecular genotyping

Genomic DNA was extracted from peripheral blood lymphocytes and tissue samples by digestion of proteinase K, followed by extraction of isopropanol and precipitation of ethanol. We used Taqman chemistry-based, pre-designed, assays for genotyping in a 7900 HT real-time polymerase chain reaction system according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA), and Sequence Detection System (SDS) version 2.1 software (Applied Biosystems) to analyse end-point fluorescence using the allelic discrimination technique. All assays were made in 384-well plates. Each plate included controls with no DNA, and genotyped samples were regenotyped on independent plates to ensure agreement of results. Any sample in which a genotype could not be accurately assessed was regenotyped.

Statistical analysis

All the reported analyses were made with the help of the online SNPStats web tool for analysis of single nucleotide polymorphisms, which is available on the website of the Catalan Institute of Oncology, Spain, at: http://bioinfo.iconcologia.net/SNPstats.[18] Analyses such as

Table 1
Details of the single nucleotide polymorphisms (SNP) analysed.

Name of gene	SNP	Alleles	Variants in cases (%)	Site	Type of SNP
Cyclin D1-1	rs647451	C/T	18.4	Chrom 11	Intron
Cyclin D1-2	rs9344	A/G	30.1	Chrom 11	Silent mutation
Cyclin D2	rs3217901	A/G	22.5	Chrom 12	Intron
Cyclin D3	rs1051130	A/C	18.0	Chrom 6	Missense mutation
Cyclin E1	rs1406	A/C	23.4	Chrom 19	3′UTR
Cyclin H	rs3093816	A/G	18.4	Chrom 5	Intron
Cyclin-dependent kinase-5 (CDK-5)	rs2069459	A/C	13.7	Chrom 7	Intron
Cyclin-dependent kinase inhibitor (p27, Kip1)	rs34329	A/G	26.7	Chrom 12	Intron
Retinoblastoma 1-1	rs3092904	A/T	20.6	Chrom 13	Intron
Retinoblastoma 1-2	rs12430215	C/G	17.3	Chrom 13	Intron