Microsatellite instability and loss of heterozygosity in human pterygia

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Abstract

***Aims/background*—Pterygium is a common benign lesion of the corneoconjunctival limbus. Although environmental factors, such as ultraviolet irradiation, have been suggested as the main causative factor in the development of the disease, however, the aetiopathology of pterygium remains obscure. In this study the possibility of detecting genetic alterations in the microsatellite DNA of the pterygium was investigated.**

***Methods*—Fifteen specimens were assessed for loss of heterozygosity (LOH) and microsatellite instability (MI) by seven microsatellite markers on four chromosomal arms.**

***Results*—Nine (60%) pterygia exhibited genetic alterations. Eight specimens (53%) exhibited LOH, while two specimens (13%) MI in at least one marker. 17q11.2-q21 is a commonly deleted region, as the frequency of LOH at this region is significantly high (47%).**

***Conclusion*—This finding indicates the existence of tumour suppressor genes in this region implicated in the disease without excluding the presence of other tumour suppressor genes in the other chromosomal regions that were examined. MI was apparent in only a few specimens but it is indeed a detectable phenomenon, suggesting that decreased fidelity in DNA replication and repair may be associated with the development of pterygium. Detection of LOH and MI, two events taking place in tumour cells or in premalignant cells, constitutes strong evidence that there must be transformed cells in the pterygial tissue and it should be considered to be a neoplastic benign lesion.**

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Pterygium is a chronic condition characterised by the encroachment of a fleshy, triangular portion of the bulbar conjunctiva onto the cornea. It is perhaps the most obvious of the ophthalmo-helioses (sun related eye conditions) and can blunt sight in several different ways.[1] Pterygium occurs in the interpalpebral fissure. It consists of an epithelium of the conjunctival type which covers a highly vascularised structure of blood vessels and loose fibrous connective tissue. It is usually growing on the nasal side of the eye and is often bilateral.[2] The exact pathogenesis of pterygium is not yet clearly understood. The most common theory concerning the origin and pathogenesis of pterygium describes the association of the disease with environmental factors such as dust, smoke, and ultraviolet irradiation. At the subcellular level, recent studies on a wide spectrum of eye and skin diseases under the ultraviolet radiation exposure, have recognised that particular molecular alterations such as the activation of oncogenes,[3 4] the aberrant expression of growth factors,[5] as well as the presence of herpesviruses in pterygium, play an important role in the development of the disease.[6] The aforementioned observations led to the suggestion that the pterygium possesses similarities with neoplasia and thus it should be considered to be a neoplastic benign lesion.

The identification of novel tumour suppressor genes (TSGs) provides information for the molecular pathway of cancer development. The inactivation of TSGs plays a critical role in multistage carcinogenesis.[7] At present, loss of heterozygosity (LOH) using highly polymorphic microsatellite markers is the most common methodology employed for the localisation of sites in the genome with high probability for the presence of candidate TSGs. Also, a recently discovered feature of the neoplastic cells is the elevated mutational rate which is reflected in the instability of microsatellite DNA.[8] Microsatellite instability (MI) has been detected in almost all human tumours[9–12] as well as in neurodegenerative diseases,[13 14] in human atherosclerotic plaques,[15] and in spontaneously aborted embryonic tissues.[16]

The aim of our study was to investigate the incidence of LOH and MI in pterygium. If indeed the molecular basis of the disease is similar to the development of neoplasia, then the above phenomena should be detectable in this lesion and might also be associated with the inactivation of TSG(s) and the presence of transforming oncogenes.

Materials and methods

TUMOUR SPECIMENS AND DNA EXTRACTION

The pterygium specimens were obtained from the ophthalmological department, University Hospital, Heraklion, Crete. The specimens were stored at −70°C for DNA extraction directly after the dissection. A matched normal DNA control either from archival sections or blood was analysed. All the specimens corresponded to primary pathological tissues. DNA was extracted as previously described[17] and stored at 4°C until polymerase chain reaction (PCR) amplification.

Table 1 Genomic instability in 15 pterygia tested with seven microsatellite markers

	Microsatellite markers						
Patient No/sex	*17q D17S250*	*17q THRA1*	*17q D17S855*	*17q D17S579*	*10q D10S109*	*11p HRM*	*8q MYC*
1/F	H	LOH	LOH	H	H	N	H
2/M	MI	H	H	H	LOH	H	LOH
3/F	–	H	–	H	H	N	N
4/M	H	H	–	H	H	H	H
5/F	H	H	LOH	H	H	N	N
6/M	H	H	H	LOH	H	LOH	H
7/M	H	H	H	H	H	N	H
8/F	LOH	LOH	–	H	N	N	N
9/F	–	LOH	H	H	H	N	H
10/F	H	H	LOH	–	H	N	N
11/M	H	H	–	H	H	H	H
12/F	H	H	H	H	H	MI	N
13/F	LOH	LOH	LOH	H	H	H	H
14/F	H	–	H	H	–	N	H
15/M	H	H	H	–	H	N	N

H = heterozygosity; LOH = loss of heterozygosity; MI = microsatellite instability; N = non-informative; — = no data.

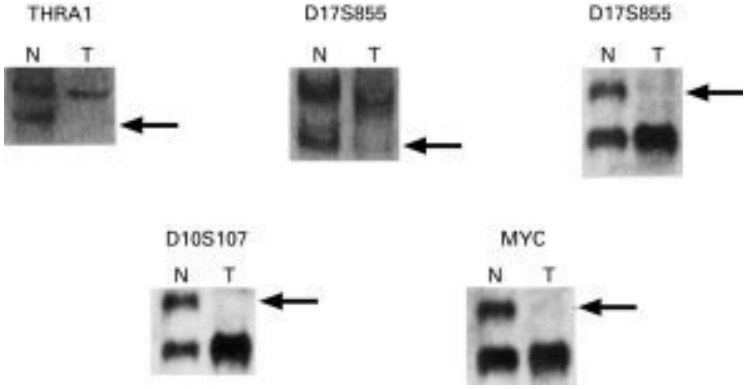

Figure 1 Representative examples of loss of heterozygosity (LOH) detected in pterygium. N = normal DNA; T = tumour DNA. Arrows indicate the position of a deleted allele. The faint bands in the position of the deleted alleles are interpreted as contamination by the adjacent normal tissue.

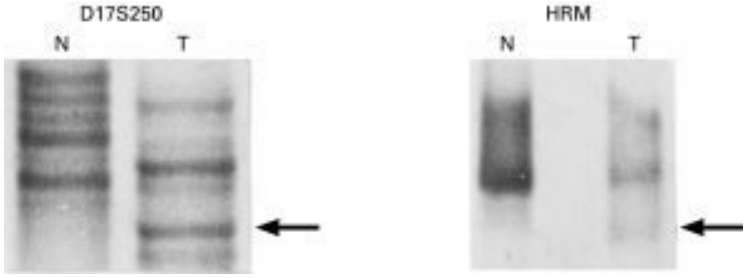

Figure 2 Representative examples of specimens exhibiting microsatellite instability (MI). N = normal DNA; T = tumour DNA. In all cases a shift in the mobility of the microsatellites is obvious and thus the specimens were scored as positive for MI.

PCR AMPLIFICATION, MICROSATELLITE, AND LOH ANALYSIS

The DNA samples were examined for genetic alterations at seven different microsatellite loci in 15 pterygia, using a bank of seven highly polymorphic microsatellite markers. Two of the microsatellite markers lie proximal to *BRCA1* (D17S250, THRA1), D17S855 lies within the *BRCA1*, D17S579 lies distal to *BRCA1*,while HRM marker is located in the first intron of *H-ras* proto-oncogene at 11p15.5. Markers MYC and D10S109 are located on chromosomes 8q24.12-q24 inside the c-*myc* oncogene and 10q11.2-qter respectively.

PCRs were performed in a 50 μl reaction volume containing 200 ng of genomic DNA, 1 μM of each primer, 250 μM dNTPs, 5 μl of 10X buffer (670 mM TRIS.HCl, pH 8.5; 166 mM ammonium sulphate; 67 mM magnesium chloride; 1.7 mg/ml BSA; 100 μM β mercaptoethanol, and 1% (w/v) Triton X-100) and 1 U of *Taq* DNA polymerase. The reactions were denatured for 5 minutes at 95°C and the DNA was subsequently amplified for 35 cycles at 95°C, 57°C, and 72°C each step. Ten μl of the PCR product were electrophoresed in a 10% polyacrylamide gel and silver stained. MI was scored by comparing the electrophoretic pattern of the microsatellite markers amplified from the paired DNA preparations that corresponded to the pathological tissue with adjacent normal tissue, demonstrating a shift of one or both of the alleles in the pathological DNA specimen. The shift was indicated by either an addition or deletion of one or more repeat units resulting in the generation of novel microsatellite alleles. The analysis in the MI positive cases was repeated at least twice and the results were highly reproducible. Gels were scanned and the intensity of the bands corresponding to the microsatellite alleles was quantitated by an image analysis system. Allelic losses were scored as decreases in intensity of one allele relative to the other as determined from comparison of pathological and normal DNAs.

Results

Fifteen surgically excised pterygia were assessed for microsatellite instability and LOH at 7q, 10q, 11p, and 17q arms. Our results are summarised in Table 1. Representative examples of specimens with LOH and MI are shown in Figures 1 and 2 respectively. Nine of 15 (60%) pterygia exhibited genetic alterations, either MI or LOH. Eight specimens exhibited LOH (53%), while two specimens exhibited MI (13%) in one microsatellite marker. Specimen No 2 exhibited MI for the D17S250 and specimen No 12 showed MI for the HRM marker (Table 1). The highest incidence of LOH was found for the marker D17S855 (4/11, 36%). Comparably high LOH was also found for the THRA1 marker. Among five cases with LOH on either of these two markers (case 8 was omitted since no data are available for D17S855), in three cases one of these markers was retained. Thus, we may postulate that the hot spot for the deletion is a gene or genes between these two markers. No sample with homozygous deletions of the region was found. Three specimens (1, 8, and 13) exhibited partial deletions of 17q which are probably due to multiple mitotic recombination events. No sample exhibited deletions of all informative markers tested in chromosome 17, indicating loss of the whole 17q arm. 17q11.2-q21 is a commonly deleted region, as the frequency of LOH at this region is significant high (47%). The age of the patients ranged between 36 and 72 years. Interestingly, LOH on this chromosomal arm was more frequent in female than in male patients.

Discussion

Recent data exist on molecular alterations in eye and skin diseases, including the activation of oncogenes[3 4] and the aberrant expression of growth factors.[5] Furthermore, the presence of herpesviruses in pterygium has been described and possibly associated with the pathogenesis of the disease.[6] However, genetic analyses in the microsatellite DNA of pterygium have not been performed and we found it interesting to investigate the relation of microsatellite DNA and the aetiopathology of the disease. In our study we describe two genetic alterations in pterygium microsatellite DNA; loss of heterozygosity indicating the presence of TSG(s), affecting 53% of the specimens and microsatellite instability corresponding to an elevated mutational rate, affecting 13% of the cases.

Genetic alterations like microsatellite instability have been detected in almost all human tumours, as well as in other diseases such as human atherosclerotic plaques,[15] leading to the suggestion that these diseases possess similarities with neoplasia and thus they should be considered as neoplastic benign lesions. Pterygium is thought to be a common benign lesion of the corneo-conjuctival limbus. Considering that pterygium cannot produce metastasis in other organs, we could classify it, from a clinical viewpoint, among benign lesions which do not progress towards malignancy. However, the cells it consists of, or a specific type of cell, display many characteristics of a tumour phenotype, with the most important of them being the continuous cellular proliferation. In this study we report the existence of another essential malignant feature—the incidence of loss of heterozygosity in the cells of pterygium. LOH is an event that takes place in tumour cells or in premalignant cells which progress towards malignant ones. This fact constitutes strong evidence that there may be transformed cells in the pterygial tissue. Most recent studies support the theory that pterygial fibroblasts have acquired many of the properties of the transformed phenotype.[18] According to Knudson's 'two hit hypothesis',[19] the phenomenon of LOH is correlated with the existence of tumour suppressor genes implicated in the disease.

A significant incidence of LOH was found for 17q11.2-q21 suggesting that important TSGs for the development of pterygium may be located on this chromosomal region. Deletions at 17q occur frequently in a variety of neoplasms. These include ovarian tumours (flanked by THRA1 and D17S75)[20]; oesophagus between probe C117-316 and C117-710,[21] laryngeal tumours between D17S250 and D17S579,[22] non-small cell lung,[23] and prostate[24] are also associated with deletions at 17q near the *BRCA1* region. The wide spectrum of human cancers affected by alterations of the candidate TSG(s) of 17q, suggests a significant role for these genes in the development of neoplasia. *BRCA1* TSG exhibits significant incidence of LOH at the pterygium. Although initial studies recognised a role for the *BRCA1* gene in the development of familial breast/ovarian cancer only, a role of this gene in the development of other sporadic tumours should also be considered. This is the first report to our knowledge on the incidence of the *BRCA1* locus deletions in a set of pterygia. LOH at the *thra1* gene is also a frequent event implicating the α receptor of thyroid hormone in the aetiopathology of the disease.

The high incidence of LOH reported in the present study indicates that TSG(s) may be located very close between the *BRCA1* and the *thra1* genes, or the inactivation of *BRCA1* and the *thra1* genes themselves may play an important role in the genesis or development of the pterygium. Fine mapping of this area is required in order to establish the precise location of these candidate TSG(s) and their role in the pathogenesis of pterygium.

The microsatellite analysis revealed an additional genetic alteration in pterygium DNA, the microsatellite instability corresponding to an elevated mutational rate. Although the number of the specimens is quite small, we detected a considerable incidence of MI. Examining the specimens with additional markers might increase our figures. The precise significance of these findings remains obscure because the information as regards the genetic basis of the disease is limited. However, we may postulate that the relatively high mutational rate of the pterygium, as reflected in the instability of the microsatellite sequences, indicates a destabilisation of the genome which may affect other genes resulting in the dysregulation of the cells harbouring these mutations. The latter may be associated with the proliferation of fibroblasts that is induced in the pterygium. This is also in agreement with the detection of possible transforming TSG(s), according to the aforementioned LOH, which is indirect evidence of a mutator phenotype. It would be of interest to screen DNA repair genes for mutations in pterygia exhibiting MI and investigate whether these mutations are also present in the germline of the patients. The latter may provide clues for the hereditary basis of the disease and is consistent with the observation that DNA repair deficiency may occur in phenotypically normal cells.[25]

MI is an early event in DNA repair deficient associated diseases. We suggest that MI, if indeed associated with the development of pterygium, is involved in the induction of the mitotic rate in pterygial fibroblasts. Future studies involving the evaluation of the clinical significance of this phenomenon as well as the molecular mechanism and consequences of MI may provide clues to the pathogenesis of the disease.

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