Mutation screening analysis of the retinoblastoma related gene RB2/p130 in sporadic ovarian cancer and head and neck squamous cell cancer

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Aims: To investigate the involvement of the RB2/p130 gene in the pathogenesis of sporadic ovarian cancer in addition to head and neck squamous cell carcinoma (HNSCC).

Methods: Paired tumour and patient matched normal DNA samples from 43 sporadic ovarian tumours and 39 normal/tumour HNSCC DNA samples were screened. The mutation screen used polymerase chain reaction (PCR) amplification followed by single strand conformation polymorphism analysis and direct sequencing of the PCR products. Exons 19 and 20 (B domain) and exons 21 and 22 (C-terminus) were analysed for mutations. These exons were chosen because most of the point mutations in RB2/ p130 are located in the C-terminal region and mutations in these exons have been identified previously in nasopharyngeal carcinomas and primary lung tumours.

Results: No abnormal band shifts were seen in the samples analysed, and no bands directly sequenced revealed the presence of mutations.

Conclusions: Genetic alterations in the RB2/p130 gene (exons 19–22) are unlikely to be involved directly in the pathogenesis of sporadic ovarian cancer or HNSCC.
They also reported mutations leading to abnormal protein localisation in lymphoid cell lines through disruption of the NLS, in addition to providing strong evidence to support the role of RB2/p130 as a tumour suppressor gene in lung cancer.

“All three retinoblastoma gene family members display growth suppressive activities, which result in the blocking of cells in the G1 phase of the cell cycle”

Cytogenetic studies have permitted the definition of chromosomal areas commonly displaying loss of heterozygosity (LOH) in tumour cells. A previous study of ovarian cancer suggested the involvement of one or more tumour suppressor genes on several chromosomes, in particular, chromosome 16q, the location of the putative tumour suppressor RB2/p130. The role of this gene in the pathogenesis of ovarian cancer was suggested by frequent LOH (38–67%) at this chromosomal region. Recently, it was reported that 17% of HNSCCs also showed LOH at chromosome 16q. In addition, Claudio and colleagues found mutations in RB2/p130 in a significant proportion of nasopharyngeal carcinomas, a subset of HNSCC.

To investigate a possible role for the RB2/p130 gene in the pathogenesis of sporadic ovarian and head and neck cancers we undertook a mutation screen analysis of the RB2/p130 gene from exons 19 to 22 in these cancers.

MATERIALS AND METHODS

DNA samples

Ovarian tumours and patient matched normal DNA sample pairs (43 pairs) were obtained from the Washington University School of Medicine (St Louis). Patient samples represented a wide range of tumour types and stages (stages I to IV epithelial tumours including 13 serous, eight endometrioid, eight mixed histological, four teratomas, three mucinous, two granulosa, two unknown, one clear cell, one fibroadenoma, and one unclassified sex cord). None of the family histories suggested a hereditary component in these patients. Normal/tumour pairs from patients with HNSCC (39 pairs: 18 laryngeal, 12 pharyngeal, seven oral cavity, and two paranasal sinuses), which included a full range of tumour stages and had been analysed for known clinicopathological parameters, were collected at the Queen Elizabeth Medical Hospital (Birmingham, UK).

PCR and mutation analysis

Tumour and normal DNA pairs were amplified by the polymerase chain reaction (PCR) using previously published primers. Exons 19, 20, and 22 of RB2/p130 were amplified using identical PCR programmes, which comprised one cycle of five minutes at 95°C and 38 cycles of 30 seconds at each of 95°C, 55°C, and 72°C, followed by one cycle of seven minutes at 72°C. Standard Taq polymerase was used for these PCRs (Gibco/Life Technologies, Paisley, UK). For PCR analysis of exon 21, a proofreading Taq polymerase (DyNAzyme; Flowgen) was used as follows: one cycle of denaturation at 95°C for five minutes, 35 cycles consisting of one minute at 95°C, one minute at 55°C, and one minute at 72°C, followed by one cycle at 72°C for five minutes (for exon 21 multiple bands were obtained with standard Taq polymerase so proofreading Taq polymerase was used).

For mutation analysis, 2 µl of each PCR product was mixed with 2 µl of loading buffer and denatured for 10 minutes at 96°C. The samples were then run in a cold chamber on 8% non-denaturing single strand conformational polymorphism (SSCP) polyacrylamide gels (Accugel 19/1; National Diagnostics, Hessle, Yorkshire, UK) containing 5% glycerol for 16–18 hours at 160–180 V, followed by silver staining and drying of the gels on to Whatman paper. Direct sequencing was performed on an ABI 377 automated sequencer using a dRhodamine cycle sequencing kit (PE Applied Biosystems, Warrington, UK).

RESULTS

Analysis of the RB2/p130 gene in ovarian cancer and HNSCC

To investigate whether mutations in the RB2/p130 gene could play a role in sporadic ovarian cancer and HNSCC, we analysed a total of 82 tumour/normal DNA pairs for mutations in exons 19–22 of this gene. Exons 19–22 span the region that encodes the B domain and C-terminus of the protein where the putative NLS is located. These exons were chosen on the basis of previous work, which identified mutations resulting in either frameshifts following insertions or point substitutions in nasopharyngeal carcinoma, or abnormal localisation of the Rb protein following disruption of the NLS in lymphoid cell lines. All four exons were amplified by PCR and were first analysed on an SSCP gel (fig 1). No abnormalities were seen upon gel analysis. Positive controls were not available for the SSCP analysis; therefore, we then directly sequenced the samples to ensure that we had not missed any shifts on SSCP as a result of false negativity. No changes were found upon direct sequencing of the exons (all ovarian samples were sequenced for exons 19–22, a third of head and neck samples were also directly sequenced). Thus, no mutations were found in the exons analysed for the 43 ovarian and 39 HNSCC tumour samples.

DISCUSSION

The importance of the Rb pathway of cell proliferation regulation is underlined by the finding that most sporadic human cancers carry mutations either in Rb or in one of the other key components of the pathway. RBl is the prototype tumour suppressor gene. In humans, retinoblastomas can arise after...
Take home messages

- No abnormal band shifts were seen in the samples of ovarian and head and neck squamous cell carcinoma analysed.
- and no mutations in the RB2/p130 gene (exons 19–22) by direct sequencing.
- Thus, genetic alterations in the RB2/p130 gene (exons 19–22) are unlikely to be involved directly in the pathogenesis of sporadic ovarian cancer or head and neck squamous cell carcinoma.

It must be mentioned that the SSCP technique does not identify all of the mutations that may be present. However, many of our samples were directly sequenced and still no mutations were found. We conclude that mutations in exons 19–22 of the RB2/p130 gene are not an important factor in the pathogenesis of these cancer types and that other as yet unidentified genes located on chromosome 16q may play a more important role.

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REFERENCES