Abstract. Melanomas are malignant tumours with high metastatic potential. The genetic alterations which lead to the transformation and progression of melanocytes to malignant melanoma remain obscure. Mutations in the ras gene family have been described, however their role in melanoma pathogenesis is still controversial. In this study we examined the incidence of H-, K- and N-ras mutations in 47 DNA samples isolated from paraffin-embedded 25 cutaneous and 22 uveal malignant melanoma tissues and a MeWo melanoma cell line using the Restriction Fragment Length Polymorphism (RFLP) analysis of PCR products. Only one mutation in codon 61 of the N-ras gene was found suggesting that the importance of ras mutations in melanoma tumorigenesis may be limited.

Introduction

Carcinogenesis is considered to be a complex multistep process (1,2). Evidence from epidemiological studies in man and biochemical and histopathological observations of tumours in vitro and in vivo suggest that chemicals, radiation and viruses are involved in the initiation of the malignancy. These factors could alter, directly or indirectly, the expression of specific genes, namely oncogenes and oncosuppressor genes and affect the machinery regulating normal growth, differentiation and programmed cell death, leading to malignant transformation (2).

The ras gene family is frequently found to be deregulated in tumours. It consists of many related genes which share the characteristic of signal transduction. Among them, the H-ras, K-ras and N-ras are the most well-characterized and studied (3). The three genes encode for highly homologous proteins (p21 ras) which are localized at the inner side of the cell membrane and present GTPase activity.

Genetic defects characterizing the ras genes include amplification and loss of a normal ras allele, but the commonest alterations involve point mutations in codons 12, 13 and 61 of the H-, K-, and N-ras genes.

The selective growth advantage conferred by ras gene mutations has been demonstrated in cell systems in vitro and mouse models. Chemical carcinogens have been shown to induce skin papillomas in mice with high percentage of activated H-ras (4) while transfection of early passage rodent cells with the mutant H-ras leads to malignant conversion by causing multiple metastasis when injected in nude mice (5,6). Furthermore, mutations in the ras genes have been described in a large range of human tumours (7,8).

Melanomas are malignant tumours with high metastatic potential. Both intrinsic and extrinsic risk factors have been discussed and studied as a possible etiology for the initiation and development of this type of tumour (9). Genetic factors may play a role, since differential incidence of melanomas has been found in different ethnic groups and family history can be a risk parameter. Furthermore, extrinsic factors, such as ultraviolet (UV) radiation, are considered as major causes of melanomas (10).

The role of ras oncogenes in the pathogenesis of malignant melanomas remains paradoxical (11,12). The incidence of ras mutations in particular is a subject of debate due to controversial data that emphasizes the confusing nature of ras gene alterations and the melanoma tumorigenesis.

In order to examine this question we studied the incidence of H- and K-ras mutations in codon 12 and of the N-ras mutations in codon 61 using the Polymerase Chain Reaction (PCR) followed by Restriction Fragment Length Polymorphism (RFLP) analysis in 47 melanomas and a MeWo human melanoma cell line.

Materials and methods

Clinical specimens and cell lines. The 22 paraffin uveal melanoma blocks were obtained from the Institute for Cancer Research Foundation, 48 Vas. Constantinou Avenue, Athens 11635, Greece; The CRC Trials Unit, University of Birmingham, Queen Elizabeth Hospital, Birmingham B15 2TH, UK; Medical School, University of Crete, Heraklion, Greece; Institute for Cancer Studies, Medical School, University of Sheffield, Sheffield S10 2RX, UK; Medical School, University of Thrace, Alexandroupolis, Greece; Ophthalmic Sciences Unit, Medical School, University of Sheffield, Sheffield S10 2RX, UK

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Figure 1. Schematic illustration of the detection of (a) H-ras codon 12, (b) K-ras codon 12 and (c) N-ras codon 61 mutations by PCR-RFLP analysis.
The conditions followed for digestions were those recommended by the supplier. Incubation temperatures were 60°C for BstNI and 37°C for MspI and MscI.

The EJ and the SW480 cells have mutant H- and K-ras alleles in codon 12 and the HL60 cell line has a mutated N-ras codon 61 allele. PCR products from amplified DNA isolated from these cell lines were used as positive controls for the completion of the digestion with MspI, BstNI and MscI, respectively.

Digestion products were electrophoresed through a 2% agarose gel (for H- and K-ras products) or an 8% native polyacrylamide gel (for N-ras products). Gels were stained with ethidium bromide and photographed on a UV light transilluminator.

Results and Discussion

The role of oncogenes in melanoma tumourigenesis is still controversial. The incidence of ras mutations, in particular, is a subject of debate. Initial studies showed that approximately 20% of cutaneous melanomas presented ras mutations, the majority of which was found in the N-ras gene (10,16). Furthermore, these mutations occurred in or adjacent to dipyrimidine sites, which is a well known target for UV damage (10). Albino et al (17) found similar results in cultured melanoma cell lines although only 5-6% of non-cultured primary and metastatic melanomas had mutated ras genes. Again, the N-ras codon 61 mutations were found to predominate. However, some reports argue the importance of N-ras in melanoma tumourigenesis. Mooy et al (18), failed to observe any N-ras mutations among the 29 DNA samples from uveal melanoma tested with asymmetric PCR and sequencing. To observe any N-ras mutations among the 29 DNA samples from uveal melanoma, O'Mara et al (19) by studying 22 melanoma short-term cell lines for N-ras mutations, identified only two, both found in codon 61 of the gene. Limited information is available on the molecular genetics of uveal melanomas. Increased myc expression has been reported and loss of heterozygosity of THRB and nm23-H1 gene have been found (20,21).

In our study, by using a PCR-RFLP (Restriction Fragment Length Polymorphism) method in 47 DNA samples, the incidence of ras mutations among the 29 DNA samples from uveal melanoma was similar to those reported by O'Mara et al (19).

Figure 2. N-ras amplification products digested with MscI and electrophoresed through an 8% polyacrylamide gel. Lane 1: Undigested positive control (HL60 cell line) (65 bp). Lane 2: Positive control digested with MscI. Lane 3: Undigested PCR product. Lanes 4-8: Negative samples (44 bp). Lane 9: Positive sample. M: pBR322/MspI DNA marker. Resistance to MscI digestion suggests a mutated N-ras gene (codon 61).
samples isolated from paraffin-embedded sections of 22 uveal and 25 cutaneous melanomas, we detected only one N-ras codon 61 mutation (Fig. 2, lane 9) in a uveal melanoma. Wild type product (44 bp) was identified in this sample, indicating heterozygosity for the mutation, which is in agreement with a previous report (19). No N-ras mutations were found in the 25 cutaneous melanomas tested suggesting that the incidence of N-ras mutations in this type of malignancy has been possibly overestimated, and similar results are shown for uveal melanomas. Uveal melanomas show very few cytogenetic abnormalities, compared with other solid tumours, and relatively little stromal tissue and cellular infiltration (22,23). The results of our study would therefore preclude a role for N-ras in the early events of tumourigenesis of melanoma.

Furthermore, we found no H- and K-ras codon 12 mutations (Figs. 3 and 4), which is in agreement with some but not all previous reports on cutaneous melanomas. A high percentage of K-ras mutations was reported by Shukla et al (24) in 40 melanotic lesions, using oligodeoxynucleotide hybridization of PCR products. On the contrary, Albino et al (25), by following similar experimental procedure, found no mutations at codon 12 in the K-ras gene. We also failed to observe any ras mutation in the melanoma cell line MeWo.

The heterogeneity of factors which could contribute to the initiation and/or the development of melanomas could partly explain the marked discrepancy in the mutation frequencies observed. Although both our study and those of others suggest that mutations in ras genes are rare in melanomas, a possible role for ras for the development and/or progression of this type of malignancy should not be excluded. Long- but not short-term expression of the viral H-ras gene has been shown to induce a complete transformed phenotype in melanocytes, possibly via introduction of chromosomal instability (26). Additional factors such as paracrine and autocrine functions and their possible interaction with the ras-mediated signal transduction may also be important. Indeed, melanoma cells produce a variety of growth factors and growth factor receptors and fibroblast growth factor (FGF) appears to have an autocrine role (27). Since ras has been shown to mediate the signal of several growth factors, including that of FGF (28), the levels of the p21 ras protein could be important. Increased levels of p21 ras protein in melanoma cells, as compared to normal melanocytes were described by Tanikawa et al (29) although Albino et al (17) found no difference in ras p21 expression between melanomas at different phases of growth or between those harboring a known ras mutation and those that did not. Finally, it has been suggested that mutated ras genes are more interactive with the machinery controlling melanocyte differentiation rather than transformation and ras gene mutations have been correlated with a subset of EGF receptor and Class II major histocompatibility (la) antigen positive melanomas from an early phase of differentiation (17).

The complexity of the up-to-date information clearly suggest that a persistent and cautious approach must be followed in order to unravel the precise role of ras gene family in melanoma tumourigenesis.

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References


