CHARACTERIZATION OF THE PROPERTIES OF AN AMPLIFIED REGION OF DNA IN PRIMARY MENINGEAL TUMORS

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Identification and characterization of all possible genetic alterations in the genome that predispose to the development of meningiomas (primary brain tumor, arising from the covering of the brain) are of importance in understanding and deciphering the exact molecular events involved in tumorigenesis. Knowledge of all genetic alterations in the genome will allow a better interpretation of the interplay between genes that are involved in the induction of meningiomas. Meningiomas are encapsulated benign tumors arising from the arachnoidal cells of the meninges, which compress and indent, but do not invade the adjacent brain or spinal cord tissue. In the cranial cavity, meningiomas are the second most common group of primary tumors. The incidence is between 12 to 20% of all intracranial tumors.

Meningiomas are generally slow-growing tumours composed of neoplastic arachnoidal (meningothelial) cells. These epithelial cells have a barrier-like function. Macroscopically, they commonly form well-circumscribed masses that have lobular architecture. Occasional tumours, particularly those lying next to the sphenoid ridge, grow in a more diffuse pattern over the dura; these are termed as meningioma en plaque. Histological grading of meningiomas is based on the current WHO classification. Most (about 90%) are WHO grade I, reflecting their benign nature. However, atypical meningiomas (WHO grade II), which make up 5-7%, and anaplastic variants (WHO grade III), 1-3%, are recognized by several histological characteristics.

Previous work in our laboratory suggests the importance of using RAPD in analyzing an overall instability in the genome under study. RAPD analysis with 10mer primer of a random but defined sequence was used to score alterations in genomes of a large population of meningiomas. The results indicated four different genetic loci were associated with meningiomas. This was due to the detection of alterations in the genome of tumor but not in the normal genome (DNA of leukocyte) of the same patient. An additional RAPD band was observed in the tumor DNA (gain of band). Based on characterization including sequencing, these were designated as *meng-1*, *meng-*

2, *meng-3* and *meng-4*. Though *meng-1*, *meng-2* and *meng-3* revealed unique hybridization patterns pointing to the possibility that these additional bands represent amplification of a set of DNA sequence exclusive to meningiomas, *meng-4* cross-hybridized with *meng-2* and vice-versa. *Meng-4* is has been shown to be a part of *meng-2.*

Characterization of such altered fragments would be the first step in understanding of the molecular mechanism leading to the onset of tumorigenesis. Hence as a part of this thesis work, a detailed characterization of the uniquely hybridizing, *meng-1* locus, has been done. The *meng-1* sequence has a very high degree of homology with the bacteriophage HK022, indicating the integration of phage sequences in the human genome. Though preliminary studies of the *meng-1* locus had been performed in the laboratory earlier, further work regarding association with the frequency of its amplification and cloning of flanking regions has been carried out. A model attempting to characterize the nature of this alteration has also been proposed. We have also studied the prevalence of these mutations in Exon-2 and Exon-11 of the *NF2* gene (a known mutation in meningiomas in this set of tumors).

In addition to genetic changes, alterations in gene expression also occur during tumorigenesis. Many cytokine receptors may also act as growth factors. Hence the expression and subunit composition of the IL-13/IL-4 receptor complex has been studied in 35 patients. IL-13 and IL-4 receptors are formed by permutations of the IL-13 family receptor subunits. Cytokines are polypeptide hormones secreted by a cell that affect growth and metabolism either of the same (Autocrine) or of another (Paracrine) cell. IL-13 is a protein secreted by activated T-cells that regulate human monocytes and B-cell functions. IL-13 shares some regulatory biological functions with IL-4. IL-13 and IL-4 gene are closely linked on Chromosome 5q23-31 and there is a sequence homology between their proteins.

OBJECTIVES OF THE WORK

In order to characterize the genetic alterations in the tumor, it was decided to concentrate on the meng-1 locus because of its marked homology with the Bacteriophage and the absence till date of any information regarding the integration of the bacteriophage sequence in the human genome (in either the public domain, CELERA, TIGR database) and there association with tumorigenesis. The percentage of tumor showing increased copy number of the *meng-1* database was studied. Experiments were conducted to identify a primer pair that reproducibly amplifies even single copy (phage homologous sequence from human DNA).

Work was undertaken to PCR amplify flanking regions of *meng-1* using primers designed from the reported bacteriophage sequence. The *meng-1* sequence has also been studied in different tumor cell. An attempt has been made to define its distribution in different population of defined ethnicity. Mutational analysis of selected exons of the *NF-2* gene has been performed.

At the level of gene expression the IL-13/IL-4receptor expression has been determined in meningiomas of different grades.

RESULTS

Of the 3 sequences shown previously, to be altered in meningiomas, the *meng-1* sequence has been studied in detail. This sequence had been shown to have a homology with bacteriophage HK022. Even in recent submissions the altered fragment *meng-1* did not show any homology to the available human sequences available in the public domain and even the CELERA and TIGR (site) domain.

4 tumors out of 35 showed increased copy number in the tumor with a primer pair, which had been designed based on the *meng-1* sequence (SCAR sequence). However, the corresponding leukocyte DNA did not show detectable amplification. This was further confirmed by Southern hybridization. The same 4 tumors showed increased copy number with an internal primer pair. 7 other tumors showed PCR products, but this was not indicative of increased copy number. Signals were mostly absent or very faint in some corre

sponding leukocyte DNA

Of the several primer pairs, designed based on *meng-1* sequence, one primer pair was identified that could reproducibly PCR amplify a stretch of DNA homologous to nt 2241 to nt 2577 of the bacteriophage HK022from all the DNA samples. The PCR product of this primer pair was present in every tumor and corresponding leukocyte DNA. The reproducibility of this PCR, reflects that the sequences chosen for priming, have been conserved, and also the ease of amplification of smaller sequences in PCR. In the 4 tumor samples that showed high copy number of *meng-1*, the intensity of the SP5-SP6 product was more in tumors, as expected. The increased amount of PCR product corresponding to *meng-1* in tumor DNA, was confirmed by multiplex PCR using *β-globulin* gene as control, indicating presence of increased copies of the *meng-1* homologous sequences in meningiomas.

Using different sets of overlapping primers based on bacteriophage HK022 sequences flanking the *meng-1* sequence we have been able to PCR amplify different stretches of phage corresponding to the tumor genome. This indicates that a large stretch of the Bacteriophage HK022 genome which is integrated within the tumor DNA. These integrated DNA sequences are present in some meningial tumors as high copy number. We have been able to PCR amplify a continuous stretch corresponding to nt 69 to nt 4202 of the bacteriophage (*meng-1* was homologous from nt 1880 to nt 3110). Genome walking was commenced commercially, from nucleotide 115 in the 5' direction. It crossed nucleotide 69 (last known nucleotide to be PCR amplified, and reached nucleotide 1 of the phage. However the next nucleotide was nucleotide 40,751 and then continued till nucleotide 40,132. This stretch therefore shows homology with both ends of the bacteriophage (linear genome). This could have been possible by recircularization before integration.

Our results indicated that single copy of *meng-1* (as shown by PCR products of primer pair SP5-SP6) is present in a battery of human tumor cell lines of different tissue origin like human glioma cell lines (U373MG, U87MG, M059 J & K), breast carcinoma cell line (MCF7), and cervical carcinoma cell line (HeLa). That different ethnic populations carried the *meng-1* sequence as was evident from nested PCR and sequencing. There is a suggestion that there may be ethnic variation in the integrated bacteriophage sequences.

For mutation detection in the *NF2* gene (Exon-2 and Exon-11) using SSCP, no change in

mobility was detected in 25 tumors. Sequencing confirmed the absence of mutations.

77% meningioma samples expressed mRNA for IL-4Ra and 100% samples expressed mRNA for IL-13Ra 1. This indicates that overexpression of IL-4 receptors shows type II structure that is an important feature of meningiomas. No IL-2γc expression was seen. The overexpressed receptors were identified by RT-PCR and confirmed in selected samples by Real Time PCR, *In Situ*Hybridization and Immuno Fluorescence Analysis. There was intra tumor heterogeneity in the overexpression of IL-4subunits. There was no relation of the overexpression with meningioma grade. There is a trend that IL-4R overexpression is more in older patients.

It is also not understood whether the IL-4 receptors have any role in tumorigenesis and signaling events in these tumors. It is possible that overexpression of these chains, especially the IL-4Ra chain, could serve as a biomarker of the disease activity and treatment monitoring for predicting response to therapy. The receptor could also be used for targeted therapy.

There were no relationship established between *meng-1, meng-2 and meng-3* with IL-13, IL-4 and its receptors.