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ORIGINAL ARTICLE

MOLECULAR CYTOGENETIC STUDY OF THE *NF2* GENE DELETION IN MENINGIOMA IN SUDANESE PATIENTS

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ABSTRACT

Meningioma is the second most common adult central nervous system tumor. Mutations and/or deletions within the tumor suppressor gene neurofibromatosis type 2 (*NF2*) are associated with meningioma development and progression. We studied 29 meningioma samples by cytogenetic analysis and interphase fluorescence *in situ* hybridization (I-FISH) using a locus-specific probe for the *NF2* gene region. We detected loss of the *NF2* gene in all samples except for one. In 10 of the 29 samples, karyotypic analyses confirmed the I-FISH results and revealed additional numerical and/or structural rearrangements in nine of them. Our study confirmed: ***i)*** the limited role of banding cytogenetics in assessing chromosomal rearrangements in meningioma, as this tumor is hard to be grown in cell culture; ***ii)*** we could show that two-color I-FISH is well-suited for NF2-deletion screening. Our results were in accordance with those of comparable studies, even though the frequency of 97.0% of meningiomas with NF2 deletions is exceptionally high in the studied Sudanese patients.

Keywords: Meningioma; *NF2* Gene; Tumor suppressor gene; Banding cytogenetics; Interphase fluorescence *in situ* hybridization (I-FISH); Sudan

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INTRODUCTION

Meningiomas are benign tumors with a relativey small number of genetic aberrations, accounting for ~1/3 of all primary brain tumors, are the second most common adult central nervous system tumors and occur in up to 1.5% of the general population; they are most frequently observed in the sixth decade of life [1-2]. Early cytogenetic studies showed complete or partial monosomy 22 as the most common chromosomal abnormality [1,3]. Later studies revealed a tumor suppressor gene on chromosome 22, sub-band q12.2, to be involved in meningioma formation and progression. Loss of the neurofibromatosis type 2 (*NF2*) gene is important in early development of meningioma [4], and plays a major role in familial meningioma which account for only ~2.0% of the cases [1-2]. Small insertions, deletion or single base pair mutations of the *NF2* gene are present in ~60.0% of sporadic meningiomas [5]. Such deletions or mutations of NF2 have been observed in >70.0% of grade II or III meningiomas, but in only 25.0% of grade I meningiomas [1]. We here report on the investigation of cytogenetic aberrations in meningioma among Sudanese patients using banding cytogenetics and molecular cytogenetics.

MATERIALS AND METHODS

Tumor Samples and Cultivation. This study was approved by the Ethical Review Board at Neilein University, Khartoum, Sudan and informed consent

was obtained from all patients. Cross-sectional design was used in this study. Twentynine meningioma tissue samples (Table 1) were collected during surgeries from the Alshaab Teaching Hospital located in Khartoum State, Sudan. Thirteen patients were male (44.8%) and 16 female (55.2%), aged 15 to 55 years, thus being much younger than meningioma patients in Western countries. Samples were collected in sterile containers with sterile RBMI-1640 media to be processed for tissue culture within two hours.

Table 1. Results obtained in 29 Sudanese Meningioma Patients.

Patient	Sex-Age	Karyotype	FISH Result (%)
1	F-40	45,XX,t(6;10)(q26;q26.2),-22	-22 (100.0)
2	F-45	45,XX,-22	-22 (80.0)
3	M-26	44,XY,-5,-22	-22 (90.0)
4	M-26	45,XY,t(5;11)(q35.2;p13),-22	-22 (100.0)
5	M-26	45,XY,del(8)(q22.2q23.3),-22	-22 (100.0)
6	M-45	44,XY,-14,t(6;10)(q26;q26.2),-22	-22 (100.0)
7	M-50	44,XY,-14,-22	-22 (100.0)
8	F-40	not available	-22 (90.0)
9	F-52	not available	-22 (70.0)
10	F-36	not available	-22 (100.0)
11	F-45	not available	-22 (70.0)
12	M-15	not available	-22 (100.0)
13	M-28	not available	-22 (90.0)
14	F-45	not available	-22 (80.0)
15	M-18	not available	-22 (100.0)
16	F-45	not available	-22 (60.0)
17	M-20	not available	-22 (90.0)
18	F-45	not available	-22 (70.0)
19	F-55	not available	-22 (100.0)
20	M-30	not available	-22 (70.0)
21	M-45	not available	-22 (80.0)
22	M-16	not available	-22 (70.0)
23	M-40	45,XY,-8,del(22)(q11q13)	del(NF2) (100.0)
24	F-40	46,XX,del(22)(q11q13)	del(NF2) (100.0)
25	F-37	not available	del(NF2) (100.0)
26	M-52	not available	del(NF2) (80.0)
27	F-38	not available	del(NF2) (90.0)
28	F-30	not available	del(NF2) (70.0)
29	M-40	46,XY	normal (100.0)

Cytogenetic and Molecular Cytogenetics. After long-term culture, chromosomes were prepared and GTG-banding was done using standard procedures [6]. Interphase fluorescence *in situ* hybridization (I-FISH) was performed using a two-color FISH approach: DNA derived from BAC-probe RP11-551L12 was *NF2* gene-specific (22q12.2) and labeled with Texas Red; RP11-172D7-DNA located in 22q11.21 served as an internal control and was labeled in SpectrumGreen. The FISH-procedure was done according to standard protocols [7]. For microscopic evaluation, 100 interphase nuclei were examined for each specimen.

RESULTS

In 10 of the 29 meningioma samples, cell cultivation led to successful karyotyping. The banding cytogenetic results are shown in Table 1.

Representative I-FISH results are shown in Figure 1. In all but one sample, deletions of NF2 were observed, in 60.0-100.0% of the interphase nuclei studied. In six of the cases the signal pattern indicated an interstitial or terminal deletion of 22q including the *NF2* gene region (Figure 1B), in the other 22 cases the signals indicated the complete loss of a chromosome 22 (Figure 1C, Table 1). Statistical analysis of the *NF2* gene deletion against age and gender did not reveal any correlation (results not shown).

DISCUSSION

In agreement with the literature, the banding cytogenetic approach led to more comprehensive results but was less successful in terms of cell cultivation and growth in almost 70.0% of the cases [8,9]. Besides chromosome 22, involvement of chromosomes 5, 6, 8, 10, 11 and 14 was observed in the present study. Even though involvement of chromosomes 8 and 10 are rather unusual findings [10], chromosome 5, 6, 11 and 14 are known to be involved in menigiomal chromosomal rearrangements [11].

In the present study, statistically there was no significant variation between gender/age and *NF2* gene deletion as initiator in tumorigenesis. This might be explained by small samples size, as previous studies demonstrated clear female predominance [12]. However, it may also be an influence of genetic

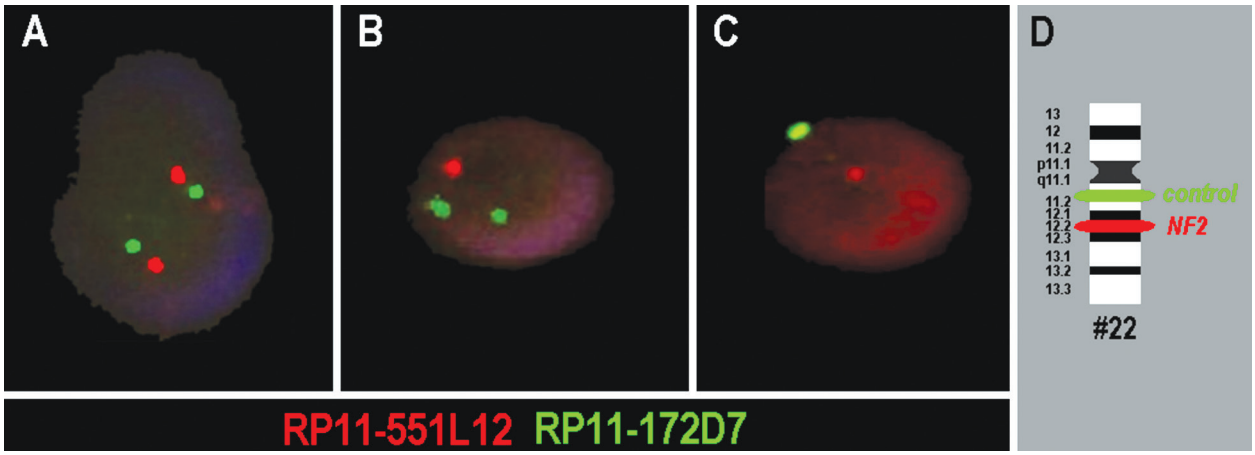

Figure 1. Typical two-color I-FISH results are depicted for three interphase nuclei of the melanoma patients. A) Two signals each in red and green were interpreted as a normal result, indicating the presence of two PROBABLY intact chromosomes 22. B) Loss of one red signal was interpreted as interstitial or terminal loss of chromosome 22q including the NF2 gene region. C) Loss of one red and one green signal were interpreted as mono-some 22. D) Schematic depiction of the probes and their localization on chromosome 22 (#22).

background of Sudanese patients; a comparable rate of 97.0% of meningiomas with NF2 deletions has not yet been found in other ethnicities. Two points that would need more comparison studies for clarification, as comparable studies found loss of NF2 only in ~60.0% of the studied patients [13]. This may have different reasons such as small sample size. However, neither an influence of ethnic background nor of young age of the studied patients (on average ~37 years) can be neglected. This study shows that even in known clinical entities more studies especially from African countries are necessary.

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