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# Hypermethylation of *BRCA1* Gene in Meningioma in Elderly Males

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**Abstract.** *Background/Aim: Breast cancer 1, early onset (BRCA1) gene is expressed in the cells of the breast and other tissues, where it plays a role in cell-cycle regulation, transcription, repair of DNA double-stranded breaks, ubiquitination, transcriptional regulation as well as other functions, such as cell response regulation to mitogenic signals triggered by estrogens. Considering that meningioma shows greater tumor growth during pregnancy, can express estrogen receptors and proliferate in response to estrogenic stimulation, the hypothesis that this type of tumor may share molecular mechanisms that involve exposure to estrogen should be investigated. Therefore, the aim of the present study was to investigate the BRCA1 gene methylation profile in meningioma. Materials and Methods: Methylation-specific polymerase chain reaction (PCR) assay was performed on 50 meningioma samples from male and female patients. Statistical analysis was carried out using Fisher's exact test. Results: The most important finding of this study was that 100% of the male patients over 55 years with meningioma showed BRCA1 methylated in their tumor cells. Conclusion: The silencing of BRCA1 through hypermethylation seems to play an important role in meningioma.*

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Meningioma are the most common tumors of the central nervous system (13 to 26% of intracranial tumors), and although they are benign and slow-growing (1, 2), they can recur after macroscopic resection (3). They are more frequent in women than men (ratio of 2:1) and show an incidence peak at 45 years of age (4).

*BRCA1* is a human gene encoding breast cancer type 1 susceptibility protein. This gene is expressed in cells of the breast and other tissues, where it helps repair damaged DNA or destroy cells if DNA cannot be repaired. BRCA1 protein associates with RNA polymerase II and, through the *C*-terminal domain, also interacts with histone deacetylase complexes. Thus, this protein plays a role in cell cycle regulation (5), transcription, repair of DNA double-stranded breaks (6, 7), ubiquitination and transcriptional regulation (8).

Estrogen, especially 17β-estradiol, is an important mitogenic agent of mammary epithelial cells (9, 10). The hormone mediates its effects by binding to its receptors, estrogen receptor alpha (ERα) and beta (ERβ) (11). Studies in knockout mice suggest that ERα has more significant roles in regulating breast development than ERβ (12). After interaction with estrogen, ER recruits co-activating proteins, forming complexes that are translocated to the nucleus and act as transcriptional factors of genes related to both proliferation and differentiation, and in other pathways of cellular metabolism (13).

BRCA1 inhibits the mitogenic effects of estrogen in mammary epithelial cells through many means, such as by repressing the transactivation function of ER by forming a complex with ER or by inhibition of P300-mediated ER acetylation, essential for its transactivation function (13-15). Furthermore, ER is a substrate of BRCA1 ubiquitin ligase activity (13, 16).

Almost half of hereditary breast cancer cases are associated with mutations of *BRCA1* (18); approximately 90% of such cases are sporadic and half of them present reduced *BRCA1* expression due to DNA methylation (18, 19).

Considering that meningioma shows increased tumor growth during pregnancy (20, 21), express ERs in approximately one-third of cases (22, 23), and proliferate in response to estrogenic stimulation (24-26), the hypothesis that this type of tumor may share molecular mechanisms that involve exposure to estrogen should be investigated.

Therefore, the aim of the present study was to investigate the *BRCA1* gene methylation profile in meningioma, in view of the fact that hypermethylation of *BRCA1* as a mechanism of inactivation of its expression has been reported for some types of tumors (27).

## Materials and Methods

The present study was approved by the Ethics Committee on Research of Sao Paulo State University (Protocol 4328-2012). Informed consent was obtained from all individual participants included in the study.

The methylation of the *BRCA1* promoter was investigated in 50 consecutive patients with meningioma (grade I) who underwent surgery at two Brazilian hospitals (Neurosurgery Service of the Sao Paulo State University Hospital in Botucatu, and of the Dr. Mário Gatti Municipal Hospital of Campinas). Among the patients, 28 were female (median age=51 years, range from 27-85 years) and 22 male (median age=54.5 years, range from 39-72 years). Seven arachnoid samples from deceased persons, unaffected by meningioma or other tumors, were used as methylation profile control. DNA was extracted from 3 $mm^3$ fragments of tumor after histopathological confirmation, using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). DNA methylation was determined by subjecting DNA to sodium bisulfite treatment, as described by Herman and collaborators (28). The modified DNA was amplified with primers specific for either the methylated (Met) or unmethylated (UnMet) sequences of *BRCA1* (Met: 5'TCGTGGTAACGGAAAGCGC3', sense and 5'AAATCTCA ACGAACTCACGCC3', antisense; unMet: 5'TTGGTTTTTGTGG TAATGGAAAAGTGT3', sense and 5'CAAAAAATCTCAACAAA CTCACACCA3', antisense). Polymerase chain reaction (PCR) was individually performed in 25 μl reaction volumes, containing 1× Platinum Taq buffer, 1.5 mmol/l$MgCl_2$, 0.2 mmol/l of each dNTP, 0.4 μmol/l of each primer set, 1 U of Platinum Taq DNA Polymerase (Invitrogen, USA) and 1 μl of treated DNA. The thermal conditions were 95˚C for 5 minutes followed by 34 cycles at 95˚C for 90 seconds, 57˚C for 1 minute and 72˚C for 2 minutes) and a final extension at 72˚C for 7 minutes. DNA methylated *in vitro* by Sss-I methylase (New England Biolabs, Ipswich, MA, USA) was used as a positive control, and water and DNA from peripheral lymphocytes of healthy donors were used as negative controls. PCR products were separated on silver-staining 6% non-denaturing polyacrylamide gels (29). Statistical analysis was carried out using Fisher's exact test, considering $p<0.05$ as statistically significant for all tests.

## Results

Sixty percent of tumors had methylated *BRCA1*, while only 14% (1/7) of arachnoid samples (controls) showed this pattern ($p=0.0394$).

According to gender, there was a predominance of *BRCA1* methylation in men (73%). In women, the distribution of methylated and unmethylated cases was even (Table I).

The methylation analysis by sex and age group (≤55 years and >55 years) showed that *BRCA1* was methylated in tumors from all men over 55 years of age ($p=0.0461$) (Table I).

Table I. *Methylation profile for breast cancer 1 early onset (BRCA1) gene in meningioma according to sex and age range.*

| | Methylated n=30 (60%) | Unmethylated n=20 (40%) | *p*-Value* |
|---|---|---|---|
| Gender | | | |
| Female | 14 (50%) | 14 (50%) | |
| Male | 16 (73%) | 6 (27%) | 0.1485 |
| Female | | | |
| ≤55 Years | 9 (50%) | 9 (50%) | |
| >55 Years | 5 (50%) | 5 (50%) | 1.3054 |
| Male | | | |
| ≤55 Years | 7 (54%) | 6 (46%) | |
| >55 Years | 9 (100%) | 0 (0%) | 0.0461 |

*Fisher's exact test; $p<0.05$ as statistically significant.

## Discussion

The most important finding of this study was that 100% of the male patients over 55 years with meningioma showed *BRCA1* gene methylation in their tumor cells. These results suggest that the lack of BRCA1 may contribute in some way to tumorigenesis in the meninges of men.

In addition to breast tumors in females, mutated *BRCA1* also increases the risk for breast and prostate tumors in males (30-32). Recent studies have found methylated *BRCA1* in tumors of both females and males (33-36).

There are discrepancies in the literature on the presence of ERs in meningioma (25, 37), perhaps due to differences in the sensitivity of the techniques employed, differences in the handling and storage of tissue, and in thermolability of ERs (38,39). However, ER has been detected in approximately one-third of these tumors (22, 23) and most cases expressed mRNA for ER (25, 40, 41). In addition, epidemiological studies have demonstrated that sex hormones may increase the risk of meningioma (42).

*BRCA1* appears to be involved in the regulatory mechanisms of the cell response to estrogen, mainly during pregnancy and puberty, when the expression of this hormone and *BRCA1* are increased (43).

In breast tumors, BRCA1 appears to act in negative modulation of the effects of both ERs and progesterone receptors (13, 44, 45), protecting the mammary tissue from any genetic instability induced by hormones through the detection and repair of DNA damage (20). Epidemiological studies have shown that the incidence of meningioma is greater in women who had breast neoplasia (20, 45, 46).

Unlike women, men show estrogen predominance in relation to their antagonistic hormones (progesterone and testosterone) after the beginning of andropause (or androgen decline in ageing males) (21, 24, 26, 46-48). This fact is corroborated by the predominance of meningioma in younger women and men older than 55 years. Associations have been indicated between hormone therapy in male trans-sexuals and increased incidence of meningioma (49).

Thus, silencing of *BRCA1* through hypermethylation appears to leave meningothelial cells subject to the mitogenic effects of estrogen. However, this hypothesis should be considered with caution because the present study did not cover the analysis of the presence of ERs in these samples.

Additionally, the lack of *BRCA1* in DNA repair might contribute to the accumulation of errors and chromosomal instability, which would increase the chances of tumorigenesis. Homozygous mutations of *BRCA1* have been associated with genetic instability in human breast epithelial cells (50, 51), however, such information is unknown in meningioma.

These findings encourage broader studies aimed at gaining a better understanding of the true role of the exposure of the meninges to estrogen in relation to BRCA1 protein expression in order to establish new therapeutic approaches in men over 55 years old with meningioma.

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