Original Article

Immunohistochemical evaluation of expression of PTEN in surface epithelial tumors of ovary

Nalini A R[1], Saravanan E[2*], Shanthi P[3]

Department of Pathology, Dr.AL Mudaliar Post-Graduate Institute of Basic Medical Sciences, University of Madras, Taramani, Chennai-600113, Tamil Nadu, INDIA.

Email: eshwar.pathos@gmail.com

Abstract

Ovarian cancer is the most common cancer to be detected in women at an advanced stage and is the fifth leading cause of cancer deaths among women globally. Molecular mechanisms in the oncogenesis of ovarian tumors remain active areas of research where PTEN, a tumor suppressor gene has been shown to mediate G1 cell cycle arrest and /or apoptosis in several cell lines such as glioma, breast, prostate and ovarian cell lines. In this study, we tried to evaluate the expression of this tumour suppressor gene and to determine the correlation of PTEN with histological sub type and grade of these tumours. PTEN mutations in surface epithelial tumours of the ovary have not been studied extensively and a thorough analysis of previous papers also reveals the possibility of a proposed area of research.

Keywords: Akt, LOH, PTEN, Ovarian tumors.

[*]**Address for Correspondence:**

Dr. Saravanan E., Department of Pathology, Dr.AL Mudaliar Post-Graduate Institute of Basic Medical Sciences, University of Madras, Taramani, Chennai-600113, Tamil Nadu, INDIA.

Email: eshwar.pathos@gmail.com

Received Date: 20/01/2016 Revised Date: 10/02/2016 Accepted Date: 14/03/2016

Access this article online

Quick Response Code:

Website: www.statperson.com

DOI: 16 March 2016

INTRODUCTION

Ovarian cancer is the most common cancer in women to be diagnosed at an advanced stage and has been the focus of extensive research with regard to identification of genes that are involved in the pathogenesis. The tumour suppressor gene PTEN/MMAC$_1$/TEP$_1$ encodes a dual specificity phosphatase and is mapped to chromosome 10. PTEN protein mediates G_1 cell cycle arrest and/or apoptosis and may play a role in cell migration and spreading. It has been shown to play a major role in the pathogenesis of a wide variety of tumours including breast cancer, gliomas, thyroid carcinomas etc. Changes in PTEN expression have been shown to underlie the development of certain histological types of ovarian cancer[1,2] particularly surface epithelial tumors of the ovary. The possible role of PTEN as a potential marker, whose relative proportions and expression has been elucidated with various types of ovarian carcinomas in this study.

MATERIALS AND METHODS

Immunohistochemical Detection of PTEN

Immunohistochemical detection of PTEN was carried out by super sensitive Non-Biotin HRP detection system (Biogenex laboratories). This detection system is claimed to achieve signal amplification and thereby an enhanced sensitivity by increasing the number of enzyme molecules which are conjugated to the secondary antibody. The primary antibody used was a Mouse monoclonal anti-human PTEN, Prediluted antibody, clone 28H6,IgG1, Kappa isotype (Biogenex Laboratories). Interpretation Of Immunohistochemistry Results The presence of a brown colour was interpreted as positive for PTEN based on the nuclear and cytoplasmic staining for PTEN. The immunohistochemistry signal was scored using the system proposed by Mohsin *et al*.[3]

Proportion Score

A proportion score was assigned to represent the estimated proportion of positively stained tumor cells. Cells were counted in 20 random fields and the percentage positivity calculated. The scoring was done by two observers and the average taken.

0 = none, 1 = less than 1/100 hpf, 2 = 1/100 to 1/10 hpf, 3 = 1/10 to 1/3 hpf, 4 = 1/3 to 2/3 hpf and 5 = more than 2/3 hpf

How to site this article: Nalini A R, Saravanan E, Shanthi P. Immunohistochemical evaluation of expression of PTEN in surface epithelial tumors of ovary. *International Journal of Recent Trends in Science and Technology*. March 2016; 18(2): 358-362. http://www.statperson.com (accessed 18 March 2016).

Intensity Score: The average estimated intensity of staining in positive cells was assigned as an intensity score.

-0 = none, 1 = weak, 2 = intermediate, 3 = strong

Immunohistochemistry Score: The proportion score and intensity score were added to obtain a total score ranging from 0 to 8. The immunohistochemistry results were classified based on the total scores:

Negative if it is 0, low positive if the score is 2 to 4 and high positive if it is between 5 to 8

A statistical analysis was carried out using the Fischer exact test. SPSS 15.0 software was used for the analysis. A p value of <0.05 is considered statistically significant.

OBSERVATIONS AND RESULTS

Of the 30 ovarian tumour samples examined for PTEN expression, 12 were benign tumours of which 6 were mucinous cystadenomas, 6 were serous cystadenomas and the remaining 18 were malignant tumours (Table 1)**.** Endothelial cells of the blood vessels were used as internal control, as they showed strong to moderate PTEN expression with predominance of nuclear staining. The intensity of the PTEN immunostaining as assessed by the IHC score in nucleus and cytoplasm of tumor cells was relatively uniform in most of the benign tumors. However, in the malignant tumors, PTEN expression differed significantly within different regions of the same tumour.

Evaluation of PTEN expression in the nucleus of the ovarian tumours (Table.1, Fig. 1)

Among the benign tumours, 25% (3/12) exhibited low positivity of tumor cell nuclei and 75% (9/12) showed high positivity for PTEN nuclear staining. Among the borderline tumours, 50% (1/2) of the tumour cell nuclei were negative for PTEN and 50% (1/2) of the tumors had nuclei with low positive IHC score. 43.8% (7/5) of the malignant tumours showed low positive pattern of IHC staining and 56.3% (9/16) expressed high positive pattern for PTEN immunoexpression. (Figs 3-6). This association of PTEN expression in tumor cell nucleus was statistically significant as the observed p value was 0.042.

Table 1: Correlation of type of tumor with expression of PTEN in tumour cell - nucleus

Type of tumor	Total number of patients	Nucleus		
		Negative(-)	Low (+)	High (+)
Benign	12	0	3(25.0%)	9(75.0%)
Borderline	2	1(50.0%)	1(50.0%)	0
Malignant	16	0	7(43.8%)	9(56.3%)
Total	30	1(3.3%)	11(36.7%)	18(60.0%)
Inference	Type of tumor is significantly associated with PTEN in tumor cell nucleus with P=0.042			

Evaluation of PTEN expression in the cytoplasm of the ovarian tumours. (Table.2, Fig.2)

33.3% of the benign tumours showed low positive IHC score and 66.7% showed high positive immunoexpression for PTEN. In borderline tumours, 50% showed negative cytoplasmic staining pattern and the rest showed low positive expression. Within the malignant tumor subset, 18.8% showed negative pattern, 43.8% showed low positive and 37.5% showed high positive cytoplasmic immunostaining for PTEN. There was no statistically significant association observed between the cytoplasmic PTEN expression and the type of tumor.

Table 2: Correlation of type of tumor with expression of PTEN in tumor cell cytoplasm

Type of tumor	Total number of patients	Cytoplasm		
		Negative (-)	Low (+)	High (+)
Benign	12	0	4(33.3%)	8(66.7%)
Borderline	2	1(50.0%)	1(50.0%)	0
Malignant	16	3(18.8%)	7(43.8%)	6(37.5%)
Total	**30**	**4(13.3%)**	**12(40.0%)**	**14(46.7%)**
Inference	**Type of tumor (Benign) shows no significant association with the expression of PTEN in cytoplasm of tumor cells with p=0.132**			

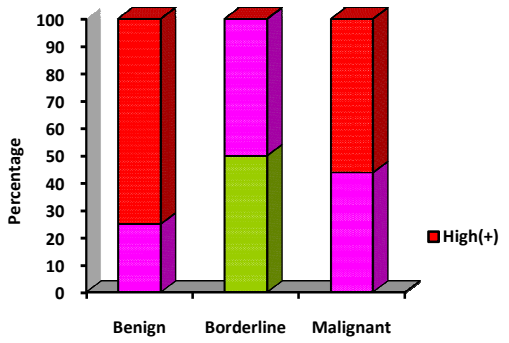

Figure 1

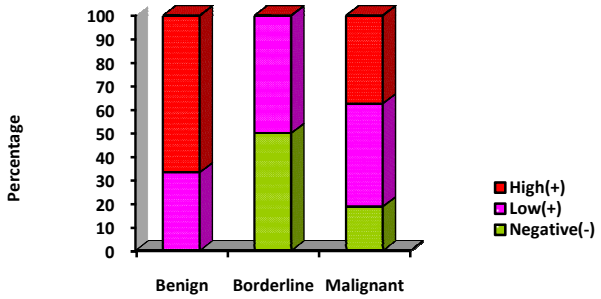

Figure 2

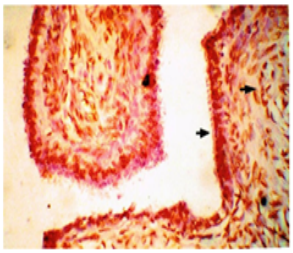

Figure 3

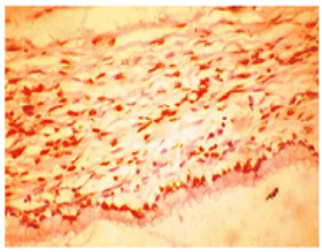

Figure 4

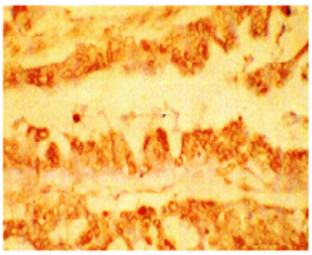

Figure 5

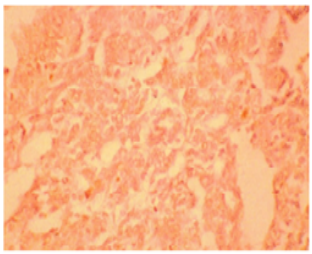

Figure 6

Legend

Figure 1: Type of tumor with expression of PTEN in tumour nucleus

Figure 2: Type of tumor with expression of PTEN in tumor cell cytoplasm

Figure 3: Cystadenofibroma - strong nuclear PTEN positivity in stromal and epithelial components X400

Figure 4: Benign Mucinous cystadenoma - nuclear positivity for PTEN X400

Figure 5: Borderline serous tumor showing low nuclear positivity for PTEN X400

Figure 6: Serous cystadenocarcinoma with tumor cells showing negative staining for PTEN X400

DISCUSSION

Cancer of ovary represents about 30% of all cancers of the female genital tract. Worldwide ovarian cancer is the sixth most common cancer in women[4]. The incidence of ovarian tumor starts increasing in the third decade and progressively increases to peak in the seventh decade. Ovarian germ cell tumors usually affect young women, with an incidence of 20 per million at 18 years age. The sex cord-stromal tumors, usually present in the 4th and 5th decades and ovarian epithelial tumors are usually found in post-menopausal women. The median age for ovarian adenocarcinomas is 60-65 years. Familial tendency, early menarche, late menopause and previous radiotherapy for benign conditions may be conducive for ovarian tumors. Other factors that have been investigated, such as talc use, asbestos exposure, high dietary fat content, and childhood mumps infection, are controversial and have not been definitively proven. Three recent studies have shown an increased risk of ovarian cancer in postmenopausal women treated with high-dose estrogen replacement therapy for 10 years of greater.[5]. Two factors consistently associated with a reduced risk of the diseases are high parity and the use of oral contraceptives. A number of specific genes have been identified as playing a role. The most important of these are BRCA 1 and BRCA 2. Majority of the familial risk of ovarian cancer is explained by BRCA 1 and to a lesser extent BRCA 2, MLH 1 and MSH 2. Ovarian cancer is a minor feature of the hereditary nonpolyposis colon cancer syndrome caused by mutation in genes associated with DNA base mismatch repair, the most frequent of which are MLH1 and MSH 2.

Tumor Suppressor Genes

Tumor suppressor genes encode for proteins whose normal function is to inhibit cell transformation and whose inactivation is advantageous for tumor cell growth and survival. The actual physiologic function of these genes is to regulate cell growth, and not to prevent tumor formation. A variety of mechanisms result in the inactivation of tumor suppressor genes, including intragenic mutations, chromosomal deletions, and loss of expression by methylation mediated transcriptional silencing or increased proteolysis. These genes participate in a variety of critical and highly conserved cell functions, including regulation of cell cycle and apoptosis, differentiation, surveillance of genomic integrity and repair of DNA errors, signal transduction, and cell adhesion.

PTEN

Phosphatase and tensin homolog deleted on chromosome 10/MMAC (mutated in multiple advanced cancers) is a candidate tumor suppressor gene located at 10q23. More precisely, the PTEN gene is located from base pair 89,613,174 to base pair, 89,716,381 on chromosome 10. Myers *et al*. establish a link between the P13K/AKT pathway and human cancers via defects in PTEN[6]. Normally AKT activity is low in the absence of growth factor stimulation. However PTEN deficient tumor cell lines as well as immortalized fibroblasts and tumors derived from PTEN deficient mice, exhibit high basal levels of AKT phosphorylation. Consistent with the anti-apoptotic actions of AKT PTEN/- fibroblasts are resistant to multiple pro-apoptotic stimuli. Reconstitution of wild-type PTEN expression restores normal AKT regulation and sensitivity to these stimuli. Da-Ming Li *et al*. in their work in human glioblastoma cells, showed that the growth suppression activity of PTEN was mediated by its ability to block cell cycle progression in G1 phase[7]. These studies suggest that the PTEN tumor suppressor modulates G1 cell cycle progression through negatively regulating the PI 3- kinase /AKT signaling pathway.

PTEN in ovarian tumors

Loss of heterozygosity (LOH) at locus 10q23 and mutation of PTEN tumor suppressor gene occurs frequently in both endometrial carcinoma and ovarian endometroid carcinoma[8,9]. Nakako Sato *et al*. in their study, found LOH at 10q23 in 42.1% of ovarian endometrioid carcinomas, 22.3% of the clear cell carcinomas and 56.5% of the solitary endometrial cysts[10]. They conclude that inactivation of the PTEN tumor suppressor gene is an early event in the development of ovarian endometrioid carcinoma and clear cell carcinoma of the ovary. Obata *et al*. (1998) in their study on 81 ovarian tumors of different histological subtypes found that LOH was common among the endometrioid (43%) and serous (28%) tumors but was infrequent among the other histological subtypes[2]. They concluded that frequent somatic PTEN mutation in endometroid ovarian tumors indicates that it plays a significant role in the etiology of this subtype. The absence of mutations in other histological subtypes indicates that epithelial ovarian tumors arise through distinct developmental pathways. PTEN expression might be a favorable biologic marker and useful prognostic indicator in patients with ovarian tumors. We have studied PTEN expression in 30 cases of surface epithelial tumours of the ovary including benign, borderline and malignant lesions. Surface epithelial tumours constitute 45% of ovarian neoplasms and more than 90% cases of ovarian cancer. We used a scoring system similar to the one used by Yasunaga *et al*. for scoring β-catenin positivity[11]. The scoring system of Kurose *et al*. was not used because there was no normal ovarian tissue in the sections used in this study. PTEN was expressed in the nucleus and cytoplasm of benign tumours, 75% of these showing high positivity of the nucleus and 66.7% in the cytoplasm. The association of high nuclear positivity with benign tumours (including both mucinous and serous) was statistically significant. In contrast, borderline tumours showed decreased expression of PTEN as compared to benign tumours – 50% of borderline tumours show low positivity and 50% high positivity. However, there were only 2 cases in this category. Malignant tumours showed decreased PTEN expression of nucleus and cytoplasm as compared with benign tumours with 60% of cases exhibiting high positivity in the nucleus and 36.7% low positivity, 3.3% being negative. This highlights the fact that decreased PTEN expression may be responsible for the progression of some benign tumours to borderline or malignant and selected borderline tumours to frank malignancy as corroborated by Sato *et al*.[10]. Many authors have reported that reduced expression of PTEN are associated with abnormalities of the PTEN gene like LOH, mutautions, deletions and epigenetic silencing.[11,12,13]. Kurose *et al*. reported decreased or absent expression of PTEN in 78% of 49 ovarian surface epithelial carcinomas[1]. The higher percentage of cases with decreased PTEN expression reported by them may be due to the inclusion of 30 endometroid carcinomas in their study. Obata *et al*. found the frequency of decreased expression of PTEN is reported to be higher in endometroid carcinomas of the ovary, as compared to other histopathological subtypes of surface epithelial tumours[2]. Our results are similar to that of Sato *et al*. who reported aberrations of the PTEN gene in 39% of ovarian carcinomas and 50% of serous carcinomas[10]. These genetic aberrations lead to decreased PTEN expression, as reported by others. The pattern of PTEN positivity in our series was both cytoplasmic and nuclear. However there was a predominance of nuclear staining in most of the cases. The nuclear staining was found to be more intense along the nuclear membrane. Localization of PTEN within the cell has been found to be cell cycle dependant, with higher levels in the G_0-G_1 phase and lower levels in the S phase, in normal cells. Interestingly, it has also been found that specific mutations affecting PTEN phosphorylation produced strong nuclear distribution of this protein[14]. The frequency of reduced PTEN expression has been reported to increase significantly with advanced clinical stage and poor outcome[15] in cancer of the breast[16] and with increasing histological grade in gliomas[17]. However, in our study, there was no significant correlation of PTEN expression with histological grade. Clinical follow up data was not available for any of the cases included in our

study. In conclusion, the majority of tumours with reduced PTEN expression were malignant, suggesting that in at least a subset of ovarian tumours, PTEN inactivation is an early event and may play a role in tumorigenesis. A combination of various mechanisms is responsible for the inactivation of PTEN pathway. PTEN may also play a role in determining the clinical course and behavior of the ovarian tumours. These issues need to be addressed by more extensive research in the future.

CONCLUSION

Ovarian neoplasms have poor prognosis due to their various histomorphological presentations and mixed tumorigenesis. Lack of insight into its etiopathogenesis is compounded by the fact it presents in an advanced stage and precursor lesion cannot be identified with ease. In this study, the expression of PTEN in varying grades of surface epithelial ovarian tumours was analyzed and it was found that in a majority of benign tumours, there was significantly increased expression of PTEN with a concomitant reduction of expression in malignant tumors. Thus PTEN inactivation may play a role in the pathogenesis of some malignant surface epithelial tumours of the ovary. Future studies on a larger series of these tumors and correlation with clinical follow up data may throw further light on the role of PTEN in prognostication of ovarian tumors.

REFERENCES

1. Keisuke Kurose, Xiao – Ping Zhou, Tsutomu Araki et al. Frequent loss of PTEN expression is linked to elevated phosphorylated Akt levels, but not associated with p27 and cyclin D1 expression in primary epithelial ovarian carcinomas, American Journal of Pathology 2001; 158: 2097 – 2106.
2. Obata KI, Morland SJ, Watson RH, Hitchcock A, Chenevix- Trench G, Thomas EJ and Campbell IG. Frequent PTEN/MMAC mutations in endometrial but not serous or mucinous epithelial ovarian tumours. Cancer Res 1998; 58(10): 2095-7
3. Mohsin SK, Weiss H, Harighurst et al. progesterone receptor by immunohistochemistry and clinical outcome in breast cancer. A validation study. Mod Pathol. 2004; 17: 1545-54.
4. Boyle P, Massonneuvre P, Autier P-Towards cancer control in women. J epidemiology biostastics 1998; 3: 137 –168.
5. Garg pp, Kerlikowske K, Subak L, Grady D. Hormone replacement therapy and risks of ovarian epithelial carcinoma : a meta analysis. Obst Gynec 1998; 92: 472-79
6. Myers MP, Pass I, Batty IH, Vandeer Kaay J, Stolarov JP, Hemmings BA, Wigler MH, Downes CP, and Jonks NK. The lipid phosphatase activity of PTEN is critical for its tumour suppressor function. Proc Natl Acad Sci USA. 1998; 95(23): 13513-8
7. Da Ming Li, Hong Sun. PTEN/MMAC1/TEP1 suppresses the tumorigeneicity and induces G1 cell cycle arrest in human glioblastoma cells. Cancer Res. 1998; 95(26): 15406-15411.
8. Jiang X, Morland SJ, Hitchcock A, et al. Allelotyping of endometriosis with adjacent ovarian carcinoma reveals evidence of common lineage, Cancer Res 1998; 58:1707-1712.
9. Gallion HH, Guarino DA, De Priest PD. Evidence for unifocal origin in familial cancer. Am.J.Obstet.Gynecology 1996; 174:1102-1108.
10. Sato N. Tsunada H, naguchi M. Loss of 10q 23.3 and mutation of tumor suppressor gene PTEN. Possible sequence progression from benign endometrial cyst to endometroid carcinoma and clear cell carcinoma of the ovary: Cancer res 2000; 50: 7052 –7056.
11. Masafumi Yasunaga, Yoshihiro Ohishi, Yoshinao Oda, Munechika Misumi, Atsuko Iwasa et al. Immunohistochemical characterization of mullerian mucinous borderline tumours: possible histogenetic link with serous borderline tumours and low-grade endometrioid tumours, Human Pathology 2009; 40, 965 – 974.
12. Gimm O, Perren A, Weng Lp, Marsh DJ, Meh JJ, Ziebold U, Gil E, Hinze R, Delbridge L, Lees JA, Mutter GL, Robinson BG, Komminoth P, Dralle H and Eng C. Differential nuclear and cytoplasmic expression of PTEN in normal thyroid tissue, and benign and malignant epithelial thyroid tumors. Am J Pathol. 2000; 156(5):1693-700.
13. Mitsuo Tachibana, Muneaki Shibakita, Satoshi Ohno, Shoichi Kinugasa et al. Expression and prognostic of PTEN product protein in patients with oesophageal squamous cell carcinoma, Cancer 2002; 94: 1955 – 60.
14. Sarah M. Planchon, Kristin A. Waite and Charis Eng. The nuclear affairs of PTEN, Journal of Cell Science 2007; 121, 249 – 253.
15. Shikha Bose, Allison Crane, Hanina Hibshoosh, Mahesh Mansukhani, Lynn Sandweis, Ramon Rarsons. Reduced Expression of PTEN correlates with Breast Cancer Progression. Human Pathology 2002; 33: 405-409
16. Peter L. Depowski, Seth I. Rosenthal, Jefferey S. Ross. Loss of expression of the PTEN gene protein product is associated with poor outcome in breast cancer, Mod Pathol 2001; 14(7): 672 – 676.
17. Rasheed BK, Mclendon RE, Friedman HS, Fuchs AE, Bidner DD and Bigner SH. Chromosome 10 deletion mapping in human gliomas : a common deletion region in 10q25. Oncogene 1995; 10(11): 2243-66

Source of Support: None Declared
Conflict of Interest: None Declared