Neuroendocrine Differentiation in Malignant and Low Malignant Potential Epithelial Tumors of the Ovary

ÖVERİN MALIGN VE DUŞUK MALIGNITE POTANSİYELLİ EPİTELYAL TÜMÖRLERİNDE NÖROENDOKRİN DİFFERANSİASYON

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Summary

Purpose: Neuroendocrine differentiation has been reported in various non neuroendocrine organ tumors such as lung and prostate tumors. However there are only a few studies dealing with neuroendocrine differentiation in epithelial ovarian neoplasms. The influence of neuroendocrine differentiation on the prognosis is not clear. The objective of this stttdv is to evaluate the frequency of neuroendocrine differentiation in malignant and low malignant potential ovarian tumors, and to find out iff here is any difference between the tumors with neuroendocrine differentiation and the tumors which do not show this finding , in terms of tumor stage.

Method: 20 malignant and 5 borderline tumors were stained with neuroendocrine markers: Neuron Spesific Enolasc, Chromogranin A. Calcitonin and Serotonin primary antibodies by streptavidin biolin immune peroxidase method.

Results: Thirty five percent of malignant and fifty percent of tumours with low malignant potential were positive with at least one neuroendocrine market: Among malignant tumours, the tumour stages were higher in tumors with neuroendocrine differentiation and showed statistically significant difference when compared with the stages of the tumors with negative neuroendocrine markers.

Conclusion: Neuroendocrine differentiation may be another factor related to stage and may be an account on to prognosis in ovarian Invasive epithelial tumors.

Key Words: Ovarian neoplasms, Neuroendocrine differentiation

T Kim J Gynecol Obst 1998, 8:97-101

Özet

Amaç: Akciğer ve prostat tümörleri gibi değişik organların tümörlerinde nöroendokrin diferansiasyon bildirilmiştir. Epitelyal över tümörlerinde nöroendokrin diferansiasyon ile ilgili çalışına sayısı sınırlıdır. Nöroendokrin diferansiasyonun prognoz üzerine etkisi belirgin değildir. Bu çalışmanın amacı överin malign ve düşük malignité potansiyelli epitelyal tümörlerinde nöroendokrin diferansiasyon sıklığını saptamak ve nöroendokrin diferansiasyon gösteren ve göstermeyen olguları evre yönünden karşılaştırmaktır.

Yöntem: 20 malign ve 5 bordeıiine över epitelyal tümörü streptavidin biotin immünperoksidaz yönlenil ile Nöron Spesifik Eıiolaz, Kromogranln A, Kalsitimin ve Serotonin primer antikorları ile boyanmıştır.

Bulgular: Malign olguların %35'inde ve borderline olguların %5 'inde en az bir nöroendokrin belirleyici ile olumlu boyanma elde edilmiştir. Malign tümörlerde nöroendokrin diferansiasyon gösteren olgularda evrenin nöroendokrin diferansiasyon göstermeyen olgulara göre anlamlı derecede yüksele olduğu izlenmiştir.

Sonuç: Överin invaziv epitelyal tümörlerinde nöroendokrin diferansiasyon evre ile ilişkili ve progıozla ilgili bir başka faktör olabilir.

Anahtar Kelimeler: Over neoplazileri, Nöroendokrin diferansiasyon

T Klin Jinekol Obst 1998, 8:97-101

Neuroendocrine (NE) cells , described by Pearse 20 years ago, are distributed widely throughout the body. These cells may form discrete organs or microscopic collections in many organs like lung, thyroid, breast, prostate , gastrointestinal system and uterine cervix (1). Although previously it was thought that these cells were not present in female genital tract apart from uterine cervix in normal conditions, there are now some reports against this assumption (2). NE cells have mem-

Geliş Tarihi: 20.01.1997

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branc bound inlracytoplasmic dense core granules ultrastructurally. and they can secrete regulatory peptids, amine products as well as enzymes like col in esterase and non-specific esterases. Thus cells which contain col in esterase and amines were called NT cells by Pearse , expressing their neural crest origin . Bui she current evidence suggests endodermal origin (1).

NF. differentiation can be demonstrated by immunohistochemistry or electron microscopy in non-neuroendoenne appearing carcinomas of the organs such as lung, prostate, thyroid and intestines which normally contain NE cells. (3-5).

The influence of the existence of neuroendocrine differentiation in the biologic behaviour of non -NE tumours is not clear. A better response to hormone treatment for prostate cancers with NE differentiation and to chemotherapy for non - NF. lung cancers with NE differentiation have been reported (3,4). However these findings were not supported by some other studies (6).

Primary carcinoid tumor is the ovarian NE tumour, and it is thought to arise from preexisting NE cells. Other than the primary carcinoid tumor and the carcinoid component of mucinous carcinomas and teratomas, there are a few reports of mucinous and endometrioid carcinomas with NE differentiation (7) . However there is noi much information regarding the frequency and the prognostic implication of NE differentiation in non-NE ovarian epitelial neoplasms.

In this study. 25 ovarian tumors consisting of 20 malignant and 5 low malignant potential (LMP) epithelial neoplasms were evaluated for the presence and the frequency of NE differentiation. The possible relation of NE differentiation to the stage of the tumor was investigated .

Materials and Methods

Surgical specimens of 25 ovarian tumors ineluding 20 malignant and 5 LMP tumors , diagnosed between 1989 and 1994 were collected from the files of our department. Cases were typed according to WHO classification and staged according to TNiv! system (7). Sections from paraffin embedded specimens were taken on poly-L-Lysinc coated slides. The tissue sections were incubated with Neuron Spesilie enolase (NSE) (DAKO NSE,H14), Chromogranin A (DAKO Chromogranin A Dak-A3), Serotonin (DAKO-5HT-H209) and Calcitonin (DAKO) monoclonal antibodies in. 1/100, 1/100, 1/10 and 1/200 dilutions respectively and immunostained by the use of streptavidin biotin immunopcroxida.se method for neuroendocrine differentiation. Diaminobenzidm trihydrotctrachloride was used as chromogen. Lung tumor sections known to be positive with these antibodies were used as positive controls. Omitting the primary antibody during staining procedure, served as negative control.

Cytoplasmic staining of the tumor cells with any of the antibodies were considered positive. The extend of the staining was scored semiquantitatively as +, if a few cells were stained, -H-, if groups of cells were stained and +++-, if there was extensive staining.

The incidence of the NE differentiation in malignant and L M P tumors were scperatcly evaluated. Tumors with and without NE differentiation were compared statistically according to their T N M stage by Kruskal Wallis test.

Results

The distribution of malignant tumors according to histologic types were as follows: 6 were endometrioid carcinomas, 6 were mucinous cystadcnocarcinomas, 7 were serous cystadenoearcinonras and 1 was undifferentiated cacinoma . The L M P tumors consisted of 3 serous and 2 mucinous tumors. The distribution of malignant tumors according to stage were as follows: 6 cases were stage 1, 2 were stage 2 and 12 were in stage 3. While 4 of borderline tumors stage 1, 1 tumor was stage 3.

The, stage and NE marker immunorcactivity of malignant and borderline tumors are shown in Table 1 and Table 2. Seven of 20 malignant tumors (35%) were stained with NE markers (Figure 1).

Table 1. NE marker staining according to the stage in malignant cases.

Stage	NÜ (r)(%)	N E (-) ("•,,)
I (n=6)	5 (25)	i (?)
2 (n=2)	2(10)	0
3 (n=12)	6 (30)	6 (30)
Total (n=20)	13(65)	7(35)

Table 2. NE marker staining according to the stage in borderline cases.

Stage	NE (A	NE (-)
1 (n-4)	1	3
3 (n=l)	I	0

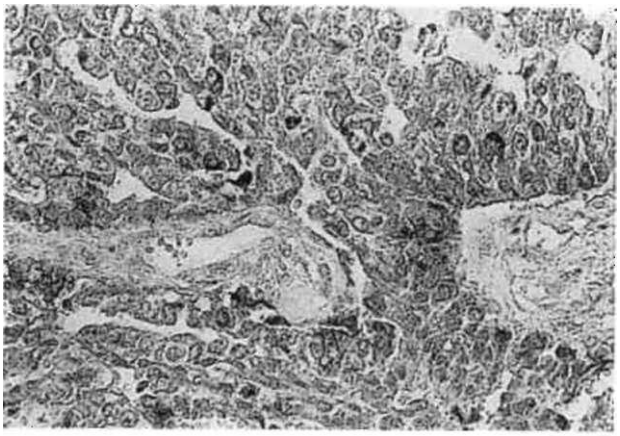

Figure 1. Cytoplasmic staining (arrow head) with Chromogranin A antibody in a serous papillary cystadcnoearcinonia (200X).

Among the malignant tumors, 2 of 6 endometrioid carcinomas (33.3%), 3 of 7 serous carcinomas (42.8%) and 2 of 6 mucinous carcinomas (33.3%) were stained with at least one marker. Three of 5 borderline tumors were stained with one or more marker, consisting of 2 serous and 1 mucinous tumors (Tabic 3) (Figure 2).

Table 3. NE marker staining according to the histological type in malignant and borderline cases.

Histological Type	NE (+) (%)	NE (-) (%)
Invasive group		
SCAC (n=8)	3 (15)	5 (25)
MCAC (n=5)	2(10)	3 (15)
EC (n=6)	2(10)	4 (20)
UC(n=-i)	1 (5)	0
Borderline group		
BSCA (n()3)	2 (40)	1 (20)
BMCA (n-~2)	I (20)	1 (20)

EC: Endometrioid carcinoma
MCAC: Mucinous cystadcnoearcinonia
SCAC: Serous cystadcnoearcinonia
UC: Undifferentiated carcinoma
BSCA: Borderline serous eystadenoma
BMCA: Borderline mucinous eystadenoma

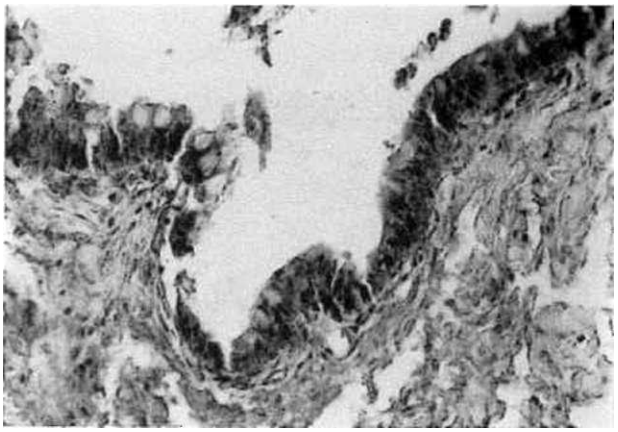

Figure 2. Diffuse cytoplasmic Calcitonin expression in a borderline mucinous tumor (200X).

There was a statistically significant difference between the stages of invasive tumors with and without NE differentiation (p=0.002), but not in borderline tumors (p>0.05). NE differentiation was observed more frequently in high stage tumors in invasive group.

Discussion

Ovarian cancer is the leading cause of death from gynecologic malignancies. Because of their rapid growth rates, and lack of early symptoms, the overall prognosis of ovarian carcinomas is poor. The 5 year survival rate is only 37%. Clinical staging is the most important factor among the factors known to influence the prognosis of ovarian cancers. Other prognostic factors include patient's age, presence of ascites, tumor's histologic type and grade, DNA ploidy, and CA 125, p 53, and c -erb - B2 expressions (7).The number of our cases in our study is low and most of them are lost in follow up . Therefore , we compared our results with tumor's stage the most important prognostic factor.

It's not surprising to find NE differentiation in non-NE carcinomas of organs and tissues which normally contains NE cells such as gastrointestinal tractus, prostate, pancreas and tyroid (3-5). Although , until recently only the uterine cervix was thought to have NE cells in the female genital tract, a recent ultrastructural and immunohistochemical study showed the precencc of NE cells in normal ovaries as well (2) . Primary ovarian carcinoid which is not a component of mucinous carcinoma or teratoma is the only NE carcinoma of

ovary. Besides, this primary NE tumor and the presence of NE component of mucinous carcinoma and teratomas , there are a few reports of mucinous cystadenomas, cystadcnocarcinomas and endometrioid carcinomas with NE differcntation in ovaries. The occurence of NE differentiation in mucinous carcinomas was reported to be more common in low malignant potential neoplasms (7) .Although the histogenesis of primary carcinoid tumor is far from to be clear, it is suggested that it arises from preexisting NE cells (2) . On the other hand , carcinoid component of the ovarian teratomas is thought to arise from the NE cells of the respiratuar or gastrointestinal epithelial components, whereas the carcinoid component of mucinous tumors is suggested to derive from metaplastic changes of surface coelemic epithelium (2) . Likewise there is not any universally accepted hypotheses explaining the occurence of NE differentiation in non NE epithelial neoplasms. While some investigators suggest that the NE differentiation in non NE tumors represent a neoplastic transformation of preexisting NE cells, others consider it a metaplastic change of coelomic surface epithelium in a manner analogous to the suggested occurence of transitional metaplasia in Brenner tumor or intestinal metaplasia in mucinous tumors (8). It is obvious that more advanced studies are needed in order to clarify embriogenesis of NE cells in ovary, as well as histogenesis of NE differentiation in ovarian carcinomas.

In this study , we demonstrated NE differentiation in 35% (7 of 20 cases) of malignant and in 60%> (3 of 5 cases) of L M P tumors. Although the number of cases in L M P group is too small to make a firm assertion , higher percentage of cases with NE differentiation in this group is in accordance with a previous report (7). Besides, it is shown here that NE differentiation occurs not only in mucinous and endometrioid carcinomas but in serous carcinomas as well . The ratio of cases with NE differentiation in endometrioid , mucinous and serous carcinomas were 33%>, 33‰ and 42.8%>, respectiveiy-

Currently there isn't a universal agreement on the type and number as well as the specifity and sensitivity of NE markers which are used to show NE differentiation in non-NE tumors. While some investigators required immunoreactivity for at least 4 or more markers, others have accepted positivity with 3, 2 or even 1 NE marker as sufficient evidence for demonstration of NE differentiation in non-NE tumors (3,9,10) . Although most of the NE markers other than N S E are considered to be rather spesific for NE differentiation, they are not sensitive .In some studies which accept sufficiency of 1 marker positivity, markers other than N S E were used (3). However in an electron microscopic (EM) study of Wilson et al, it was shown that all of the tumors with dense core granules detected by E M , showed immunohistochemically positive reaction with N S E while others without dense core granules failed to do so, indicating that N S E might indeed represent NE differentiation (11). Besides it has been suggested in some studies that positivity with N S E in large number of tumors might be due to its sensitivity for detection of cells with minimal NE differentiation.(i.e. those with few secretory granules or low levels of hormones) that can not be detected by other tecniques (12). In our study , only 1 out of 10 positively stained cases was reactive only with N S E , but the staining reaction was diffuse and strong. A11 the other 9 cases were stained either with 2 or more markers or with markers other than NSE.

There are some conflicting reports on the influence of NE differentiation in the prognosis of non NE carcinomas of lung and prostate. While some studies suggested that NE differentiation was a favourable independent prognostic factor in lung and prostate non - NE carcinomas , others did not support these findings (3,4,6). In this study, because of the lack of sufficient follow up data, we investigated the relation of NE differentiation to the stages of those non-NE ovarian epithelial tumors with and without NE differentiation. We found a statistically significant difference between the malignant tumors with NE differentiation and those without such differentiation, , in terms of tumor's stage (PO.05). The malignant tumors with NE differentiation were found to be in higher stages. Such a difference was not observed in L M P tumors , but the number of cases in this group is to small for a comment.

Although , the malignant tumors included in this study are heterogeneous, consisting of different types and their numbers are too small to reach a

more assertive conclusion, it may be suggested that NE differentiation might be another factor related to stage and an account of it to the prognosis in invasive ovarian tumors and it might be worthwhile to investigate the prognostic value of NE differentiation in larger scries with survival data.

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