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REVIEW ARTICLE

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Serous tumors of low malignant potential of the ovary-molecular pathology: part 2

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Abstract A progressive development from serous tumors of low malignant potential (SLMP) to bluntly invasive serous carcinoma has been suggested in parallel to the concept of adenoma–carcinoma sequence in colorectal carcinomas. However, recent genetic data enforces a reassessment of the concept that SLMP tumors represent precursor lesions to invasive serous carcinoma. Despite the benign nature of the majority of these tumors, some will behave worse. The identification of those SLMP tumors with an aggressive clinical behavior remains difficult, regardless of a growing body of molecular pathologic investigations. Expression of p53, c-*erb*B2, as well as the presence of *ras* mutations are not helpful in this respect. Immunostaining of both MMP-2 and basement membrane components such as collagen type IV, as well as the disintegration of collagen type I at the tumor-host interface, may be helpful for the diagnosis of a microinvasive SLMP, but it remains questionable whether this is important for prognosis. The differential diagnosis to frankly invasive carcinoma depends on the detection of destructive stromal invasion. In questionable cases, the loss of N-cadherin would argue for the presence of a carcinoma whereas the coexpression of p21 and MDM2 is rather characteristic for SLMP tumors.

Keywords Borderline tumors · Tumors of low malignant potential · Ovary · Serous tumors · Genetics · Hormone receptors · Oncogenes · Tumor suppressor genes · Inflammation · Desmoplasia

Introduction

The first part of this review was focussed on morphological aspects of serous tumors of low malignant potential (SLMP) [34]. In Part 2, we will summarize the conflicting data on both cytogenetic investigations and molecular biological aspects of SLMP tumors which have been published during the last two decades.

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Genetic alterations associated with SLMP tumors

Many studies have been performed to investigate genetic aberrations in SLMP tumors. Summarizing the cytogenetic reports, we found chromosomal abnormalities in 37% (Table 1) [23, 30, 49, 55, 62, 90, 104, 124, 125, 135, 140]. Compared to data obtained by DNA cytometry (see Part 1) describing a rate of aneuploidy of around 10%, the above mentioned percentage is surprisingly high. An explanation for this discrepancy is that the cytogenetic abnormalities in SLMP tumors are subtle. Very frequently described aberrations are trisomies of chromosomes 2, 7, and 12 [23, 55, 88, 89, 90, 91, 93, 124, 140]. However, there are also more complex patterns of chromosomal alterations as reported in the study of Tibiletti et al. [125], revealing losses on chromosome 10, 15, 17, 19, and X, as well as gains on chromosomes 1, 3, 4, and 5. The most frequent alteration in their study was a deletion on 6q12 in 5/6 SLMP tumors. Most of the data cited above were obtained by conventional cytogenetic investigation, bearing the risk of both selection and cell culture artifacts. However, genetic alterations were detected also by other methods. Using interphase cytogenetics, Diebold et al. [33] found gains on chromosomes 6 (38%), chromosome 7 (31%), and chromosome X (15%), as well as losses on chromosomes 3 (25%) and 1 (23%). Reports on losses of heterozygosity (LOH) are frequent (in 22% of SLMP tumors at one or more loci [132]) but do not reveal a homogenous pattern. A frequently involved site is the androgen receptor locus at Xq12, where LOH has been described in 23% of cases [37]. However, data on the X-chromosome are contradictory because some authors observed losses [7, 55, 90, 124, 125] while others reported gains [30,91]. In the study by Dodson et al. (1993) [35] there was a LOH on 10q in one

Table 1 Genetic abnormalities described in SLMP tumors of the ovary

Author	Number of cases	Alterations	Method
Knoerr-Gaertner et al. [62]	6	1 case without 5 cases 47,XX, +10	CCG
Crickard et al. [23]	5	3 cases without 47, XX, +2, +12, –3 45, XX, +12, –9, –14; 46,XX, +7, –9; 48,XX,+2, +7	CCG
Samuelson et al. [104]	1	47, XX, +12	CCG
Yang Feng et al. [140]	5	2 cases without 47, XX,+12 (2 cases) 46, XX,-1,-11,+r(1),+der(11)t(1;11)	CCG
Jenkins et al. [55]	3	1 case without 47, XX,+12 in 2 cases	CCG
Thompson et al. [124]	3	47, XX,+7; 48,XX,+7,+8 57–58<2n>, XX,+3,+4,+5,+7,+8,+11,+12,+14,+1,+20 46, XX,r(1)(q23p36),+8,+12,t(14;16)(q31;q13)	CCG
Pejovic et al. [91]	7	4 cases without 47, XX,+12 47, XX,+5,+8,+12 47, XX,r(1)(p36p42)	CCG
Tibiletti et al. [125]	6	1 case without 46, XX,del(6)(q25→qter) 46, XX,ins(16;6)(q25;q23q26) 37–48, XX,del(6)(q27→qter),-17,-19 36–52, XX,+3,+4,+5,del(6)(q27→qter),-10,-12,-15,-17 42–45, XX,-X,+1,del(6)(q24→qter),+1–2mar	CCG
Deger et al. [30]	4	47–49, XX,+7, +8, +mar 47, XX, +7 45–46, XX, -6 47–49, XX, +7, +7, +7	CCG
Wolf et al. [135]	9	7 cases without 2 cases gains on 5;7;8;13;14q32-qter; 15q22-qter; 20; loss on 22	CGH
Hauptmann et al. [49]	7	2 cases without 5 cases gains on 3p, 4q, 5q, 6q, 9p, 12q, 13q; losses on 1p, 8, 9q, 16p 19, 22	CGH

Table 2 Genetic abnormalities described in low-grade invasive serous carcinomas of the ovary

Author	Number of cases	Alterations	Method
Pejovic et al. [89]	1	52, XX, +5,+7, +8, +14, +21	CCG
Samuelson et al. [104]	1	47, XX, +12	CCG
Tharapel et al. [123]	1	46, XX	CCG
Pejovic et al. [90]	7	4 cases without 50, XX, +5, +7, +12, +14 46, XX, der(15)t(8;15)(q11;p13) 47, XX, der(1)t(1;1)(p21;q22); +mar, and 47, XX, -11; der(1)t(1;1)(p21; q22); del(20)(p11); +mar	CCG
Jenkins et al. [55]	1	47, XX,+12 [7]; 93,XXXX,del(1)(p22),+12×2,-16 [1]	CCG
Thompson et al. [124]	7	46, X,-X,+12 47, XX,+12 [5]j/46,XX [8] 47, XX,+12 [10]j/47,XX,+20 [3]j/47,XX,der(6)t(6;7)(p21.3;q22), del(7)(q22), +12, der(19)t(6;19)(p21.3;q13.3) [3] 47, XX,+7 [8]j/46,XX[9] 52, XX,+5,+7,+8,+8,+13,+14 47, XX,+8,+12,-15, der(19)t(15;19)(q15;p13.3) 48, XX,+7,+8,+12, dic(19;22)(p13.1;q13)	CCG
Iwabuchi et al. [54]	18[a]	Gains on 1, 3q, 5p, 8q, 11q, 12p, 13q, 18q, 20q Losses on 16q, 17, 19, Xp	CGH
Hauptmann et al. [49]	6	Gains on 1q, 3q, 6p, 8q Losses on 4q, 6q, 12q, 13q, 16q	CGH

[a] Including 50% non-serous carcinomas

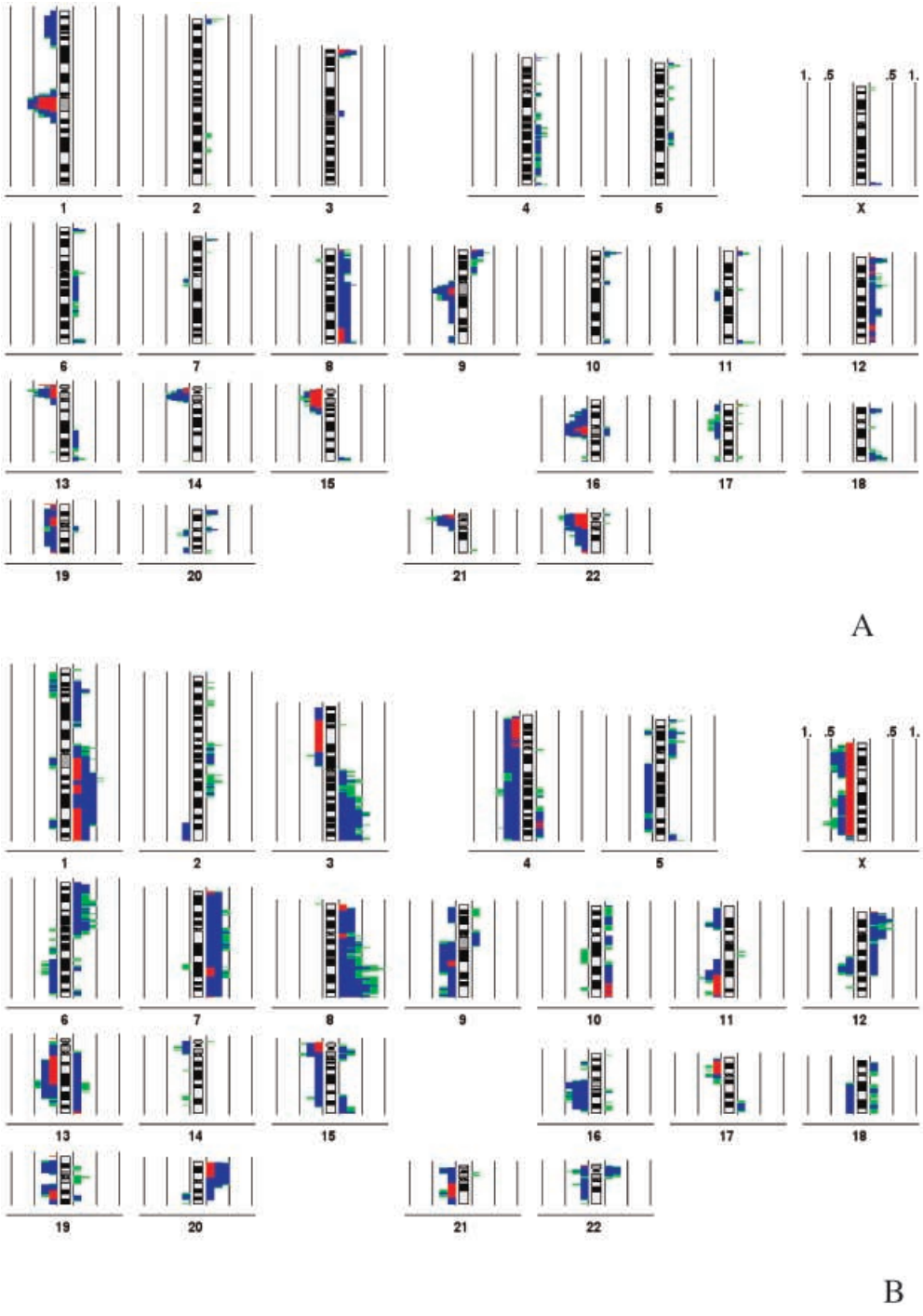

Fig. 1 Histogram of genetic imbalances of SLMP tumors **A** and low-grade serous invasive carcinomas **B** Gains (deviation to the right from the chromosome ideogram) and losses (deviation to the left from the chromosome ideogram) are marked in *green* (95% significance) or *blue* (99% significance). The *red* color indicates pronounced alterations. The *lines* parallel to the ideograms indicate the frequency of a given alteration (line closest to the ideogram=50%; most peripheral line=100%)

of four cases. LOH at the 6q25–q27 locus was found in 11% [99]. In a series of 22 SLMP tumors investigated in our laboratory [103], LOH was found in only two cases: in one case on 5q21, in the second on 17p13 and 2q21–22, but LOH on 17p and 2q was also found in two serous cystadenomas. In a recent study which includes 11 serous cystadenomas and five SLMP [141], there was a relatively high degree of LOH on 1p, 7q, 8p, 11p in both entities, and, additionally on 17q in SLMP tumors.

To find an answer to the question of whether SLMP tumors are precursor lesions of invasive serous carcinomas, it is important to compare the respective genetic aberrations. The cytogenetic data from the literature suggest that invasive serous carcinomas of a low degree of malignancy have genetic aberrations in 91% of cases (Table 2). Although trisomies 7, 8, and 12 also occur in invasive serous carcinomas of low grade malignancy, there are a lot of differences (Fig. 1), suggesting different pathogenesis from the beginning.

The initial reports on a high frequency of microsatellite instability (MI) in SLMP tumors [47,118] have to be challenged. According to the actual recommendations for diagnosing MI [11,95] there is only one case in the study of Haas et al. [47] and there are two cases in the

report by Tangir et al. [118] fulfilling the criteria, giving an incidence of MI in these tumors of 5% and 11%, respectively.

It could recently be shown that 84% of LMP tumors express the homeobox gene SIX5, which is also present in the normal epithelium throughout most of the female reproductive tract. Notably, this gene is not expressed in either adenomas or carcinomas of the ovary [134], once more indicating that SLMP tumors are a genetically different disease.

Hormone dependence

Almost all of the SLMP tumors show strong immunohistochemical expression of both estrogen and progesterone receptors, explaining early reports where in some cases the peritoneal lesions of stage III SLMP tumors have disappeared after oophorectomy or tamoxifen administration, suggesting that in these cases the process was hormone-dependent [59]. Further evidence of hormone-dependent growth of SLMP tumors comes from a report by Mooney et al. [79]. They described unusual clinical and histological features in SLMP tumors from pregnant women with marked epithelial proliferation, microinvasion, and lymph node metastasis which regressed after termination of pregnancy. This indicates that progestins may also be of some importance for the growth of these tumors. Moreover, it has recently been shown that mRNA of the FSH receptor is present in 94% of LMP tumors [144] whereas the LH receptor is present in only 24% [70]. The importance of both LH and FSH receptors for the growth of these tumors is an open question.

Oncogenes, tumor suppressor genes, and growth factor receptors

The p21 protein

The p21 protein belongs to the cyclin-dependent kinase inhibitors and modulates the cell cycle by interrupting the transition from G1 to S phase, and it is transactivated by p53 [38]. An interesting finding is the p21 positivity in more than 90% of SLMP tumors whereas adenomas and carcinomas were positive in only 37.5% and 50%, respectively [87]. Another unique finding of this study was the coexpression of p21 and MDM2 in 80% of SLMP tumors, whereas this combination was found in adenomas and carcinomas in only 26% and 28%, respectively. Because both these proteins have anti-proliferative effects [12], their overexpression could be one explanation for the low proliferative rate which is typical for these tumors.

The p53 protein

The p53 protein is a transcription factor involved in maintaining genomic integrity and control of cell cycle progression and cell survival [113]. Using immunohistochemistry, most authors did not find p53 in ovarian cystadenomas and SLMP tumors, whereas p53 positivity has been shown in 50–88% of invasive ovarian carcinomas [5, 14, 36, 53, 60, 61, 63, 72, 110, 122]. However, other investigators had described p53 immunoreactivity in SLMP tumors, ranging from 4% to 33% [10, 14, 20, 39, 64, 66, 68, 87], which is in contrast to the low frequency of LOH on chromosome 17p13 (see above). A very high p53 immunoreactivity of 65% of SLMP tumors was found by Marcelli et al. [71]. They described p53-positive tumor cells more frequently in papillary regions compared to flat areas. A comparably high frequency of p53 immunoreactivity has been described in micropapillary serous carcinoma (see Part 1), but, as in conventional SLMP tumors, there are no p53 mutations [58]. A high immunoreactivity of p53 in the absence of mutations indicates an upregulation of wild type p53. It can also be explained by the expression of the oncogene mdm2 because the protein forms a stable but inactive complex with p53 [142]. Whereas initially MDM 2 was thought to be absent in SLMP tumors [110], a more recent investigation [87] describes MDM 2 in 87% of these tumors – a percentage comparable to the situation in invasive serous carcinomas. Thus, the detection of p53 in SLMP tumors in the absence of mutations can be explained by the overexpression of MDM2.

In contrast to invasive carcinomas p53 immunoreactivity has proved to be unrelated to the clinical course of SLMP tumors [39,65]. On the other hand, Berchuk et al. [10] as well as Caduff et al. [14] found p53 positivity predominantly in stage III SLMP tumors, and Gershenson et al. [44] were able to demonstrate that p53 expression in advanced stage SLMP tumors was associated with a fourfold increased risk of progression/recurrence as well as with a decreased survival.

The p73 protein

This protein has a high sequence homology to p53. Its functional activity is perhaps different from p53 but it is not yet completely understood [73]. Whereas LOH on the p73 locus on 1p36 has been observed in invasive ovarian carcinomas, all the LMP tumors (type not specified) were heterozygous. Notably, a biallelic expression was found, and, compared to the normal surface epithelium, 92% of the LMP tumors demonstrated overexpression of the p73 transcript [84], suggesting that this protein plays a role in these tumors.

The c-*erb*B2 oncogene

The c-*erb*B2 oncogene codes for a protein ($p185^{c\text{-}erbB\text{-}2}$) with high homology to the epidermal growth factor (EGF) receptor. Amplification of the c-*erb*B2 gene and/or overexpression of its gene product has been detected in 14–76% of invasive serous carcinomas, and has

been shown to be associated with poor prognosis [9,101] whereas other studies failed to confirm its prognostic significance [22, 41, 102,112]. In cystadenomas and SLMP tumors, too, c-*erb*B2 was expressed in a comparable frequency (up to 83%) and with the same considerable range as in carcinomas [39, 57, 65, 76, 101, 108, 109]. Up to now, the c-*erb*B2 expression was not found to be helpful in characterizing SLMP tumors with an aggressive clinical course.

The *ras* family

The role of k-*ras* mutations (in particular in exon 12) in the pathogenesis of serous ovarian tumors is not clear. These mutations have been observed in 0–13% of serous cystadenomas, in 9–48% of SLMP tumors, and in 0–39% of invasive serous carcinomas [14, 21, 24, 40, 46, 65, 78, 96, 122], indicating that the frequency of k-*ras* mutations is slightly higher in SLMP tumors than in invasive serous carcinomas. A more detailed analysis by Haas et al. [46] revealed a comparable frequency of k-*ras* mutations in SLMP tumors and highly differentiated invasive serous carcinomas, but their absence in poorly differentiated invasive serous carcinomas. Therefore, they assumed that SLMP tumors and highly differentiated invasive serous carcinomas are related. Although there are data indicating that k-*ras* mutations were found predominantly in stage III SLMP tumors [14,46], there is no evidence that SLMP tumors with k-*ras* mutation behave worse than tumors without such an alteration. In this regard, it is important to note that the frequency of k-*ras* mutations in SLMP tumors is much higher than their risk of progression. Investigations of the other members of the *ras* family revealed a much lower frequency of H-*ras* mutations in both SLMP tumors and invasive serous carcinomas [128], whereas N-*ras* mutations were not found.

The BRCA1 gene

This gene encodes a protein acting in concert with DNA repair enzymes to maintain the integrity of the genome during periods of rapid growth and, therefore, has a tumor suppressor function [51, 107]. The incidence of BRCA1 germ line mutations is much higher in patients with invasive serous carcinomas than in patients with SLMP tumors [45], indicating that the probability of functional impairment of the protein is enhanced in the latter. This is in line with the finding that LMP tumors were not found in patients from BRCA1-positive families [131]. Moreover, a nuclear BRCA1 protein expression has been found in 75% of cystadenomas, in all SLMP tumors and in most of the low-grade carcinomas as well, but was completely absent in high-grade invasive ovarian carcinomas [145]. However, looking at the ratio of BRCA1-positivity to the MIB1-index, it has been shown that this ratio is comparable in adenomas and SLMP tumors but is reduced in low-grade carcinomas, suggesting a functional deficiency of the BRCA1 protein in low-grade invasive carcinomas.

Apoptosis

The apoptotic index of SLMP ranges between 0.6% and 2,8%, which is lower compared to that observed in invasive serous carcinomas, although there is an overlap [20, 32, 139]. Concerning proteins involved in regulation of apoptosis, contradictory data are present. Whereas in early studies a decreased expression of the anti-apoptotic protein Bcl-2 was described in SLMP tumors compared to the normal surface epithelium of the ovary [50, 74, 130], a recent investigation only found a slight reduction in staining intensity [20]. The pro-apoptotic protein Bax seems not to be altered in SLMP tumors [130]. However, there are currently no data on the biological significance of the expression of regulators of apoptosis in SLMP tumors. Taken together, although prognostic significance of both Bax and Bcl-2 has been shown in invasive serous carcinomas [50,74], there is no conclusive picture on the situation in SLMP tumors.

Telomerase activity

Telomerase is an enzyme which repairs the shortened chromosome edges arising after each cell division. With a few exceptions, this enzyme is either inactive or expressed with low activity in normal somatic cells, causing replicative senescence. Immortalization, one of the results of neoplastic transformation, results in re-expression of telomerase activity, which is found in nearly all malignant tumors. Concerning SLMP tumors, contradictory data have been published. In the study of Murakami et al., no telomerase activity was found in two SLMP tumors, whereas all invasive serous carcinomas showed telomerase activity [81]. This is in accordance with the observation that the human telomerase catalytic subunit (hTERT) is not present in SLMP tumors [67]. However, other investigators have found telomerase activity in all or the majority of tumors [28,103].

Tumor–stroma interactions in SLMP tumor

Invasive growth, the main differential diagnostic criterion between SLMP tumors and invasive serous carcinomas, is regulated to a large extent by interactions of the tumor cells with the surrounding stroma. The latter consists of extracellular matrix and inflammatory cells. Tumor cells themselves express various adhesion molecules facilitating migration through the connective tissue.

Inflammatory cells

Among the inflammatory cells, tumor-associated macrophages (TAM) play a pivotal role in regulation of inva-

sion. TAM can promote tumor progression by production of mediators which regulate proliferation and migration of tumor cells, stimulate angiogenesis, or contribute to the production of lytic enzymes, like urokinase-type plasminogen activator (uPA) or matrix-metalloproteases (MMPs), which enhance tumor cell invasion [110]. Although there is no detailed report on the inflammatory infiltrate in SLMP tumors, the inflammation seems to be more intense in invasive serous carcinomas than in cystadenomas and SLMP tumors [8, 48, 56, 82, 84, 98, 127]. Macrophages and CD8-positive T cells predominate in the tumors studied so far. It is interesting to note that most of the macrophages within borderline tumors express both Fas and Fas ligand, indicating an activated state [8].

Urokinase-type plasminogen activator

The serine protease uPA is produced by macrophages, fibroblasts, and tumor cells and converts plasminogen to the active, trypsin-like protease plasmin, a matrix-degrading enzyme favoring invasion of tumor cells. The presence of specific uPA receptors on the cells of epithelial ovarian tumors was shown by Casslén et al. [18]. They also found that the total number of uPA receptors was higher in carcinomas than in adenomas. It was further shown that the cytoplasmic amounts of both uPA and plasminogen activator-inhibitor-1 (PAI-1), an inhibitor of uPA, were increased from cystadenomas to SLMP tumors and were highest in invasive serous carcinomas [13, 19,115]. It would be interesting to investigate whether uPA or PAI-1 is a useful prognostic factor in SLMP tumors.

MMPs

This growing family of proteases has been shown to be produced as inactive zymogens by stromal cells and tumor cells of a variety of malignant tumors. The function of these enzymes is degradation of extracellular matrix and activation of pro-MMPs, which favors tumor cell invasion. Some of these enzymes (MMP-1, MMP-2, MMP-7, and MMP-9) have been investigated in serous ovarian tumors. The number of reactive tumors as well as the frequency and staining intensity of reactive cells within the lesions increased from adenomas to SLMP tumors and to invasive serous carcinomas [15, 31, 43]. Moreover, there is an enhanced production of MMP-2 and MMP-9 by fibroblasts and macrophages in invasive ovarian carcinomas compared to adenomas and SLMP tumors [4, 15,83]. This indicates that tumor cells of invasive carcinomas produce mediators stimulating MMP production in stromal cells. As in invasive serous carcinomas, there is a correlation between disruption of the basement membrane and MMP-2 reactivity of the tumor cells in microinvasive SLMP tumors [15,31]. MMP-7 was found in the tumor cells of both SLMP tumors and invasive serous carcinomas (85% and 79%, respectively) [120]. Because the majority of SLMP tumors express this protease, it is probably not directly involved in invasion. However, MMP-7 is able to activate pro-MMPs and, in turn, may be indirectly involved in the regulation of invasion. Another subgroup of MMPs are membrane type-matrix metalloproteases (MT-MMP) which activate pro-MMP-2. One member of this family, MT1-MMP has been investigated in serous ovarian tumors [2]. Whereas cells of serous adenomas did not express MT1-MMP, single positive cells were found in SLMP tumors. In contrast, invasive serous carcinomas had a high frequency of MT1–MMP-expressing cells [2]. A new protease with matrix degrading properties which could be identified recently in ovarian tumor cells, is hepsin. Compared to normal ovary there is a marked elevation of this enzyme in SLMP tumors, which is further enhanced in invasive serous carcinomas [119]. Another novel protease, the stratum corneum chymotryptic enzyme, is overexpressed in both SLMP tumors and invasive carcinomas (67% and 78%, respectively)[121]. Therefore, immunostaining of both MMP-2 and basement membrane components such as collagen type IV may be helpful for the diagnosis of a microinvasive SLMP tumors, but there is no data proving a prognostic relevance.

The extracellular matrix (ECM) is composed of various proteins, proteoglycans, and some other components. It is known that the composition of ECM components within the stroma of malignant tumors is different from that of the normal tissue, because their ratio has changed or because new matrix components were produced which are normally not present. Particularly the latter fact has raised the question as to whether the detection of such ECM molecules is useful as a differential diagnostic aid.

Tenascin

The glycoprotein tenascin (TN) is widely expressed during the fetal development. In the adult organism, TN has a very restricted expression pattern, but it is produced in large amounts in the stroma of various malignant tumors. Unfortunately, TN has already been found in the stroma of ovarian adenomas. The expression intensity was higher in SLMP tumors, but is highest in invasive ovarian carcinomas [133]. Moreover, various splicing variants of the mRNA of TN were found in carcinomas (types not mentioned), but not in SLMP tumors.

Vitronectin

Vitronectin (VN) is also an extracellular matrix glycoprotein that serves as substrate for cell migration, but it also interferes with the plasminogen activation system and, therefore, modulates matrix degradation. VN was found to be present in LMP tumors and highly differentiated carcinomas but not in poorly differentiated carcinomas [17].

Fibronectin

Another matrix glycoprotein present in large amounts in the stroma of malignant tumors is fibronectin (FN). Although the expression of oncofetal isoforms of FN has been shown in malignant ovarian tumors [77], no detailed study on FN expression in SLMP tumors is available.

Fibulin

Recently, the estrogen-regulated matrix glycoprotein fibulin-1 has been found in epithelial ovarian tumors. Whereas fibulin-1 staining was weak in normal ovaries, it increased in some serous cystadenomas and was markedly expressed in the stroma of papillary structures in SLMP tumors as well as in invasive serous carcinomas [100]. This matrix glycoprotein is produced mainly by the epithelial cells and interacts with FN and laminin. It probably has an inhibitory effect on cancer cell motility.

Collagens

Studies on collagen type I and type III were performed by Zhu et al. [146,147]. They analyzed the expression of newly synthesized type I procollagen and the cross-linked type I collagen which are present in the normal ovarian stroma. No changes were observed in cystadenomas and SLMP tumors, whereas in carcinomas a disintegration and destruction of collagen type I was found particularly adjacent to invading protrusions. Differing results have been published for type III collagen, the major extracellular matrix component of the ovary. Collagen type III was found continuously at the epithelial–stromal junction with tightly arranged fine fibers, whereas in SLMP tumors and invasive serous carcinomas the expression of collagen type III was decreased and more irregularly distributed.

Taking these data together, it is evident that the composition of the extracellular matrix in SLMP tumors differs from the normal ovary but stands in between adenomas and carcinomas. TN is produced to some extent but, in contrast to invasive carcinomas, no alternatively spliced isoforms occur. VN as well as fibulin-1 were found in large amounts, whereas collagen type III was decreased. Data on FN and glycosaminoglycans are not available. Whether the disintegration of collagen type I is helpful in identifying microinvasive areas remains to be clarified.

Changes in the extracellular matrix composition are regulated by a network of growth factors produced by both epithelial and stromal cells. Growth factors with a high impact of ECM production are transforming growth factor β1 (TGFβ1), fibroblast growth factor-2 (FGF-2), and platelet-derived growth factor (PDGF). Data on the expression of these molecules in ovarian tumors in general are sparse. Growth factors regulating tumor neovascularization such as vascular endothelial growth factor (VEGF) are much better investigated.

Platelet-derived growth factor

Only one study, performed by Henriksen et al., has investigated the expression of PDGF and one of its receptors (PDGF-Rα) in serous ovarian tumors [50]. Whereas the receptor could be detected in 14% of SLMP tumors and in 35% of invasive serous carcinomas, the former produced PDGF in 57% and the latter in only 7%, indicating that there are differences in the PDGF-pathway between invasive and non-invasive serous tumors.

Vascular endothelial growth factor

VEGF is a very important angiogenetic factor produced by tumor cells or inflammatory cells within the tumor stroma. It has been shown that the microvessel density in SLMP tumors is significantly lower than in invasive serous carcinomas [83]. Accordingly, an elevation of VEGF mRNA has been described in only 12–33% of SLMP tumors as compared to 49–100% of invasive carcinomas [1, 42, 88, 114,138]. Although a negative prognostic influence of elevated VEGF expression has been found even in early stage invasive ovarian carcinomas [88], there are no data proving whether VEGF is an indicator of SLMP tumors with high risk for recurrence. Whether there is a correlation between k-*ras* mutations and high VEGF expression, as could be expected by experimental data presented by Rak et al. [97], or whether SLMP tumors with p53 mutations have a decreased expression of the anti-angiogenic extracellular matrix molecule thrombospondin-1, as expected judging by data presented by Damerson et al. [25], is currently under investigation in our laboratory.

Intercellular adhesion molecules are necessary for the maintenance of tissue integrity. One group of these molecules are the cadherins, which mediate interactions between epithelial cells. Other adhesion molecules, such as integrins and CD44, mediate the interactions between cells and the extracellular matrix and are important structures for tumor cell migration.

N-cadherin

N-cadherin is present on normal surface epithelium of the ovary. Expression of N-cadherin was observed in all serous adenomas and SLMP tumors, whereas 30% of carcinomas were negative. Concerning the staining pattern, N-cadherin is homogeneously expressed in 75% of serous cystadenomas and 60% of SLMP tumors. In contrast, the staining pattern of invasive carcinoma was generally heterogeneous [92,136].

E-cadherin

E-cadherin is another member of this adhesion molecule family, which is not found on the surface epithelium of the ovaries although α- and β-catenins are present [29, 117]. However, as soon as an epithelial inclusion cyst is formed, the epithelium starts to express E-cadherin [3, 117]. The expression of E-cadherin is maintained in serous adenomas and is found in around 70% of both SLMP tumors and invasive serous carcinomas [26, 117]. However, a homogenous pattern of expression was found in 50% of both serous adenomas and SLMP tumors, but in only around 10% of invasive serous carcinomas [26, 117]. Moreover, the polarized expression of E-cadherin is lost in both SLMP tumors and invasive serous carcinomas, suggesting that there is a loss of its functional activity. This, in turn, could be the result of mutations of both E-cadherin or β-catenin but can also be induced by tyrosine phosphorylation of β-catenin [6]. Moreover, it was shown that beside cadherins there is also a competition of cadherins, adenomatous polyposis coli protein, and growth factor receptors like EGF-R and c-erb B2 for the interaction with β-catenin [52], indicating that E-cad will be regulated by a complex network.

Therefore, the expression of E-cadherin in SLMP tumors is comparable to what is seen in invasive serous carcinomas, but its functional activity remains to be clarified. While the espression of E-cadherin is not useful in the differential diagnosis between SLMP tumors and invasive serous carcinoma, the loss of N-cadherin suggests the presence of an invasive carcinoma.

Integrins

Integrins are a growing family of transmembranous heterodimeric proteins acting as extracellular matrix receptors. It is known that malignant tumors have an integrin pattern different from that of normal tissue. Integrins play a role in peritoneal seeding of ovarian tumor cells, because peptides containing the RGD peptide sequence (the recognition motif of most integrins) inhibit this process [138], and are involved in the activation of uPA pathway [80].

Ovarian surface epithelium normally expresses the integrin chains α2, α3, α6, αv, and β1, β3 as well as β4 [17,111]. There is only a limited number of studies dealing with integrin expression in SLMP tumors. An enhanced expression of αvβ3 (which binds FN and VN [16]) has been described in 50% of SLMP tumors but also in 76% of invasive serous carcinomas [69]. In the study of Carreiras et al. [17] this integrin heterodimer was found in all SLMP tumors and highly differentiated carcinomas but was decreased in high grade carcinomas (of various histology). The integrins α2β1 and α5β1 (the receptors of collagen type I and fibronectin, respectively [80]) were expressed in 64% of SLMP tumors and in approximately 80% of invasive serous carcinomas.

Table 3 Comparison between adenomas, LMP tumors, and carcinomas

Criterion	Adenomas	LMP tumors	Carcinomas
Nuclear BRCA1-positivity	75%; 6% positive cells	100%, 30% positive cells	GI-carcinomas: 82%; 21% positive cells GIII-carcinom negative
BRCA1/MIB1-ratio	1.36	1.36	GI-carcinomas: 0.72
Hormone dependence		Partly	No
p21/MDM 2-coexpression	26%	80%	28%
p53-protein	Negative	4–33%	50–88%
p53-mutations	No	No	Yes
Importance of p53 alterations		Indicates poor prognosis in stage III tumors	Poor prognosis
ras-mutations	0–13%	9–48%	0–39%
MMP2-expression in epithelial cells	Rare	Suspicious for microinvasion	Frequent
MMP2/9 in stromal cells	No	No	Present
MT1-MMP	No	Single cells	Most cells
Tenascin	Low	Intermediate	High
Vitronectin		Present	Present in GI-carcinomas
PDGF-expression		57%	7%
VEGF-expression		12–33%	49–100%
N-cadherin	100%; 75% homogenous	100%; 60% homogenous	70%; patchy
E-cadherin	100%; 50% homogenous polarized	70%; 50% homogenous not polarized	70%; 10% homogenous not polarized
CD44 s		54%	64%
CD44v3		47%	100%
CD44v6		37%	20–42%

The hyaluronic acid receptor CD44

CD44 is the main hyaluronic acid receptor and is involved in binding of ovarian carcinoma cells to mesothelial cells. Several CD44 isoforms are generated by alternative splicing of its mRNA. The CD44 standard variant (CD44 s) is expressed in scattered cells of the normal ovarian surface epithelium and increased in epithelial ovarian tumors (30%, 54%, and 64% of cells within an adenoma, SLMP tumor or serous carcinoma, respectively [27]). Splicing variants of CD44 are usually not found in the normal surface epithelium of the ovary. The variant isoforms which seem to be important in epithelial ovarian tumors are CD44v3 (present in 47% of SLMP tumors and all the carcinomas) and CD44v6 (present in 37% of SLMP tumors and 20–42% of invasive serous carcinomas). Although CD44v6 expressing SLMP tumors and invasive serous carcinomas were found to be of higher proliferative activity and were more frequently p53 positive, the biological importance of CD44 remains controversial [106,126].

Conclusion

Cytogenetic aberrations are found in 37% of SLMP tumors but in 91% of invasive serous carcinomas of low-grade malignancy. In the former the aberrations appear at random with only little overlap to the invasive carcinomas (Table 1 and Table 2). Also the other molecular data (Table 3) enforce a reassessment of the concept that SLMP tumors represent precursor lesions to invasive serous carcinoma (Table 3).

For practical purposes, the two main problems are still the differential diagnosis to invasive serous carcinoma and the selection of those cases which will take a progressive course. Despite an enormous amount of molecular data, there is currently only little help for making the decision about these important questions. The main criterion for the diagnosis of invasive carcinoma is still the identification of destructive stroma invasion. A loss or a patchy pattern of N-cadherin can be regarded as a hint, but it is not diagnostic. Expression of MMP-2 and fragmentation of basement membrane components, such as collagen type IV, are helpful in identifying microinvasion. As of yet there is no molecular marker which can identify those SLMP tumors which will behave worse. We expect progress will be made in the future in understanding the differences between SLMP tumors and invasive carcinomas of low-grade malignancy by the use of cDNA-microarray technique.

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