PHENOTYPIC CHARACTERISTICS OF PERITONEAL TUMOR IMPLANTS IN OVARIAN EPITHELIAL TUMORS: A CRITICAL REVIEW

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SUMMARY

Ovarian borderline malignancies are heterogeneous in 80-90% of cases and are characterized by a favorable prognosis, while in 10-20% of cases, peritoneal implants form and relapse occurs. The presence of peritoneal implants has uncertain predictive value. According to some authors, they undergo regression, and in some instances, long-term survival is observed despite the presence of disseminated implants. Implants are also classified into invasive and non-invasive types. Such a classification may have predictive value, so it is an active study area. According to recent studies, cytokines secreted by macrophages induce angiogenesis by ovarian tumors and evade immune surveillance. The frequency of macrophage distribution in the mesothelium may indicate disease spread and be associated with broader tumor dissemination. The role of the peritoneum in tumor dissemination processes is an active area of research. The development and metastasis of ovarian epithelial carcinoma are associated with fibrosis, one of the driving forces in the epithelial-mesenchymal transition process. Therefore, deciphering the regulators of epithelial-mesenchymal transition in ovarian epithelial tumors is necessary to develop new therapies to prevent metastatic spread and improve patient survival rates.

Thus, the correct identification of peritoneal implants is an essential factor. Although there are histological criteria to distinguish invasive from non-invasive implants, differentiation can be difficult. Additionally, little is known about the molecular-genetic basis of implants. This issue requires further research to determine diagnosis, treatment methods, and prognosis accurately.
Keywords: peritoneum; implants; microenvironment; prognostic markers; ovarian epithelial tumors.

Ovarian cancer is one of the most common pathologies among gynecological malignancies. Each year, there are approximately 210,000 new cases of ovarian epithelial carcinoma, with

128,000 resulting in death.[1] In Georgia, according to 2021 data from the NCDC, 274 new cases of malignant ovarian tumors were recorded[2].

Despite the treatment provided, the 5-year survival rate for ovarian cancer is approximately 46-49%.[1] The incidence is significantly lower before menopause and increases post-menopause, leading to an average age of diagnosis of 63 years. The risk of developing ovarian cancer is 1 in 70, but in women carrying germline mutations in the BRCA1 and BRCA2 tumor suppressor genes, the risk increases significantly.[3]

There is a dualistic model for the development of ovarian epithelial tumors, which is widely accepted. This model divides ovarian tumors into Type I and Type II groups.[4] Type I tumors include low-malignancy serous, endometrioid, clear cell, mucinous, and seromucinous carcinomas. Type II tumors include high-grade serous carcinoma, carcinosarcoma, and undifferentiated carcinoma. This group is characterized by a more advanced stage, a higher age group at diagnosis, and greater genetic instability compared to Type I. Both types of tumors differ in their origin cells, precursor lesions, and variations in molecular-genetic mutations. Intermediate precursors of Type I tumors are borderline malignant tumors, often arising from cystadenomas, whereas Type II tumors develop from serous tubal intraepithelial carcinomas of the fallopian tube. Consequently, serous borderline tumors (SBT) precede the development of low-grade serous carcinoma (LGSC). Most cases are detected in the 20-50 age group (average age 46).[4]

Although most serous borderline malignant tumors have a benign course, some cases progress to serous carcinoma, significantly increasing mortality. This process is not well understood. Some studies indicate that in 2/3 of serous borderline ovarian tumor cases, there is somatic activation of KRAS or BRAF mutations, playing an essential role in their progression.[5] This could be used as a biomarker to assess the risk of progression from borderline malignant ovarian tumors to low-grade serous carcinoma.

Ovarian borderline malignant tumors are heterogeneous in 80-90% of cases and are characterized by a favorable prognosis, whereas in 10-20% of cases, peritoneal implants form and relapse occurs. According to one study, in patients with serous borderline malignant ovarian tumors with invasive implants in the peritoneum, more than 30% showed progression to serous carcinoma.[6]

The distinguishing criterion between ovarian borderline malignant tumors and serous carcinoma is mainly the presence of stromal invasion, regardless of extrinsic ovarian existence. The presence of peritoneal implants has uncertain predictive value. According to some authors, they undergo regression, and in some cases, long-term survival is observed despite the presence of disseminated implants. Implants are also classified into invasive and non-invasive types. Such a classification may have predictive value, so it is an active study area.

There is a hypothesis that implants with invasive properties are characteristic of both ovarian serous tumors and borderline malignant tumors, and their presence may indicate disease progression. Research is limited, providing information on the phenotypic characteristics of invasive and non-invasive implants.

As noted, primary tumors of the peritoneum are rare. Secondary tumors of the peritoneum are more common and complicate the course of most intra-abdominal tumors.[7] Their prognosis depends on the nature of the primary tumor. Without intervention, the prognosis for peritoneal

carcinomatosis of any etiology is poor, with a survival rate of only a few months. Peritoneal carcinogenesis can be explained by mechanisms such as lymphatic or hematogenous spread, serous migration, spontaneous or traumatic (surgical) dissemination, and perforation.[8]

The peritoneum's characteristic structure, distinguishing it from other fat-rich visceral tissues, is its well-vascularized immune cell structures, predominantly represented by lymphocytes and macrophages and often colonized by tumor cells. Interestingly, colonization of the peritoneum by ovarian cancer cells in immunocompromised experimental mice (lacking T, B, and NK cells) occurs as successfully as in non-immunocompromised models, indicating the involvement of non-lymphoid tissues in this process.

Cytokines Secreted by Macrophages Cause Angiogenesis and Immune Surveillance Evasion by Ovarian Tumors

According to recent studies, the frequency of macrophage distribution in the omentum may indicate disease dissemination and is associated with more extensive tumor spread. However, the characteristics of ovarian tumor spread in the omentum cannot be fully explained by macrophage quantity alone, as they constitute the dominant cell population in peritoneal fluids (60%).

The role of the peritoneum in tumor process dissemination is a subject of active study. It is hypothesized that signaling pathways associated with peritoneal metastasis formation include several key molecules: 1) E-cadherin and epithelial-mesenchymal transition, which are involved in settlement of tumor cells; 2) the actin microfilament system, involved in the transport of tumor cells within the peritoneum; 3) intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1), tumor cell receptors such as CD44, and cytokines like tumor necrosis factor-alpha (TNFα), interleukin-beta, and interleukin-gamma, which facilitate tumor cell dissemination; 4) metalloproteinases and integrins, which mediate tumor cell invasion; 5) epidermal growth factor receptor (EGFR), epidermal growth factor (EGF), transforming growth factor-alpha (TGFα), insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor and its receptor (VEGF and VEGFR), which are involved in tumor cell proliferation and angiogenesis.[9]

Once tumor cells dissociate from the primary tumor site as single cells or cellular clusters, they metastasize via passive mechanisms, i.e., transported to the peritoneal surface and omentum through the physiological movement of peritoneal fluid. A significant molecule that aids tumor cells in separating from the primary site is E-cadherin. The expression of E-cadherin is significantly lower in peritoneal metastatic cells of ovarian tumors compared to cells in the primary tumor site. This fact may indicate that low E-cadherin expression confers a more invasive potential to the tumor, and the absence of its expression is associated with lower survival rates.

After dissociating from the primary tumor site, ovarian cancer cells are present in peritoneal fluid as multicellular spheroids or single cells. In tumor cell spheroids, cells maintain an epithelial phenotype and express Sip1, a regulator of E-cadherin and matrix metalloproteinases (MMP-2).[10] At this stage, integrins such as α5β1 and its ligands, fibronectin, are located on the surface of tumor cells and play a crucial role in binding to other ligands, such as α6β1 and α2β1. These molecules modify the tumor cells' microenvironment in the peritoneal ascitic fluid. The various characteristics of the microenvironment determine the interaction of tumor cell spheroids' surface receptors with the peritoneum or omentum surface.[11]

Proteolytic activity is also essential in the spread of tumor cells. Matrix metalloproteinases such as MMP14 and MMP2 may facilitate the disaggregation of tumor cell spheroids and their adhesion to peritoneal mesothelial cells.

Integrins are critical mediators in the signal transduction between ovarian carcinoma cells and the mesothelium, contributing to the spread, invasion, and peritoneal metastasis of ovarian cancer cells. Integrin αvβ6 binds to the RGD peptide, which is presented in the LAP peptide, associated with TGF-β1 as part of the latent transforming growth factor-beta binding protein 1 (LTBP1), causing conformational changes in the TGF-β1-LAP-LTBP1 complex. This complex, known as the latency-associated complex, is released by integrin αvβ6, which then binds to its receptor, activating the signaling pathway. Studies show that Wnt5A induces αv integrin expression in ovarian cancer cells, indicating a positive correlation between Wnt5A, αv, and β6 expression in metastatic serous ovarian carcinoma samples. Research also demonstrates that Wnt5A is an essential mediator in the initial stage of epithelial-mesenchymal transition in ovarian carcinoma metastasis.[11]

The development and metastasis of ovarian epithelial carcinoma are associated with fibrosis, one of the driving forces in the epithelial-mesenchymal transition process. Therefore, understanding the regulators of epithelial-mesenchymal transition in ovarian epithelial tumors is essential for developing new therapies to eliminate metastatic spread and improve patient survival rates.

The Wnt signaling pathway is critically important, and its dysregulation is closely associated with tumor progression.[12] β-catenin-independent Wnt signaling, known as the non-canonical pathway, includes the Wnt/Ca2+ and Wnt/planar cell polarity (PCP) pathways, which mediate cell polarity, movement, and cytoskeletal reorganization. Wnt5A is a key non-canonical Wnt molecule that can act as a tumor promoter or suppressor in various carcinomas. Wnt5A demonstrates tumor-enhancing effects and may be associated with epithelial-mesenchymal transition in the progression of ovarian carcinoma.[11]

The Role of TGFβ in Fibrosis and Epithelial-Mesenchymal Transition (EMT)

TGFβ plays a crucial role in fibrosis and subsequent EMT through various effects, including the Smad signaling pathway. Members of the TGFβ superfamily generate signaling pathways via type 1 and type 2 serine/threonine kinase receptors, which form a heteromeric complex.

Ovarian Tumors with Borderline Malignancy

Ovarian tumors with borderline malignancy are characterized by the absence of stromal invasion, and their primary prognostic factor is the type of peritoneal implants. These implants are considered invasive when cell proliferation involves underlying tissues (peritoneal surface, omentum, and intestinal wall) or non-invasive. Whether these implants represent metastasis from the primary site or de novo neoplastic transformation of the peritoneal surface is still unknown.[13]

Mitochondrial DNA sequencing was conducted to assess clonality in eight patients with both ovarian borderline malignancies and peritoneal implants.[13] In 37.5% of cases, similar mitochondrial DNA mutations were found in both the ovarian borderline malignancies and the implants, suggesting that the implants may originate from the primary tumor site.

Genetic and Molecular Analysis of Peritoneal Implants

Other sources suggest that peritoneal implants differ clinically and diagnostically from serous borderline ovarian tumors. Studies have been conducted to determine whether peritoneal implants and serous borderline ovarian tumors have a monoclonal origin. According to these studies, KRAS and BRAF mutations are present in two-thirds of low-grade serous tumors. However, little is known about the molecular-genetic basis of the implants.

Immunohistochemical studies were conducted to examine the presence and distribution of mesothelial cells, stromal fibrocytes, and myofibroblasts in invasive and non-invasive implants using the following antibodies: Calretinin, CD34, and α-SMA.[14] All cases of invasive implants revealed a loss of mesothelial cells and stromal fibrocytes, whereas most non-invasive implants retained mesothelial cells and stromal fibrocytes. Myofibroblast proliferation was present in all cases of invasive implants and approximately half of non-invasive cases. The loss of mesothelial cells and stromal fibrocytes in conjunction with myofibroblast proliferation was a specific indicator for differentiating invasive from non-invasive implants, providing an essential morphological diagnostic aid. According to the study above, these antibodies' combined sensitivity and specificity were 100% and 81%, respectively. However, this method may not be suitable for small biopsies of non-invasive desmoplastic implants.

Molecular Characteristics and Therapeutic Resistance

Research shows that high-grade metastases and invasive implants exhibit irregular expression of oncogenes and tumor suppressor genes, with different pathway-specific disruptions. Irregular tumor suppressor genes are enriched with DNA repair genes such as BRCA1/2 and MSH6, which are involved in developing high-grade serous carcinoma and low-grade malignant carcinoma of the ovary. Increased gene expression may result from gain-of-function mutations due to hypomethylation of regulatory regions. Reduced expression may be attributed to loss-of-function mutations or epigenetic silencing. Cell survival and proliferation may increase depending on the mechanism affecting oncogenes and tumor suppressor genes.

To evaluate the malignant potential of invasive implants, a study was conducted on genes including ABCB1, CDC2, CDKN1A, FAT1, MMP9, MSH2, NQO1, and TOP2A.[15] These genes are associated with chemotherapy resistance in ovarian cancer.[16] Additionally, ABCB1 is involved in cell migration and growth in vitro and correlates with poor prognosis in serous ovarian cancer. CDC2 and CDKN1A genes regulate the cell cycle. The FAT1 gene is a member of the cadherin superfamily and controls cell proliferation.[17] MMP9 participates in the progression of malignant tumors. It is believed to facilitate tumor progression, including invasion, metastasis, and angiogenesis, by mediating the degradation of type IV collagen in the basement membrane and extracellular matrix. NQO1 is a family member of NAD(P)H dehydrogenase (quinone). NQO1 regulates the ubiquitin-independent degradation of p53. NQO1 stabilizes p53, protecting it from degradation. Tumors with reduced NQO1 expression/activity exhibit decreased p53 stability, possibly leading to chemotherapy resistance. NQO1 is associated with poor prognosis in patients with serous ovarian carcinoma. Finally, TOP2A encodes DNA topoisomerase, an enzyme involved in DNA transcription and replication.

Implications for Diagnosis and Treatment

Proper identification of peritoneal implants is a critical factor. Despite the presence of histological criteria distinguishing invasive and non-invasive implants, their differentiation can be challenging. Additionally, little is known about the molecular-genetic basis of the implants. This issue requires further research to determine diagnosis, treatment methods, and prognosis accurately.

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