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REVIEW

Tubal and ovarian pathways to pelvic epithelial cancer: a pathological perspective

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Prolongation of ovarian epithelial cancer survival depends on early detection or improved responses to chemotherapy. Gains in either have been modest at best. Understanding the diverse pathogenesis of this disease is critical to early intervention or prevention. This review addresses six important variables, including (i) cell of origin, (ii) site of origin, (iii) initial genotoxic events, (iv) risks imposed by hereditary and other promoting conditions, (v) subsequent factors that promote different patterns of metastatic spread, and (vi) prospects for intervention. This review proposes two distinct pathways to pelvic epithelial cancer. The first initiates in ovarian surface epithelium (OSE), Mullerian inclusions or endometriosis in the ovary. The second arises from the endosalpinx and encompasses a subset of serous carcinomas. The serous carcinogenic sequence in the distal fallopian tube is described and contrasted with lower grade serous tumors based on tumour location, earliest genetic change and ability (or lack of) to undergo terminal (ciliated) differentiation. Ultimately, a clear understanding of tumour origin and the mechanism(s) leading to the earliest phases of the serous and endometrioid carcinogenic sequences may hold the greatest promise for designing prevention strategies and/or developing new therapies.

Keywords: breast cancer gene, ovarian neoplasms, serous carcinoma, tubal intraepithelial carcinoma, tubal neoplasms

Abbreviations: BRCA, breast cancer gene; MIC, Mullerian inclusion cyst; OSE, ovarian surface epithelium; PTEN, phosphatase and tensin homologue

Introduction

Ovarian cancer afflicts approximately 25 000 women in the USA each year. The causes are not well understood due to the heterogeneity of the disease and the lack of clarity regarding the initial events leading to malignancy. Moreover, a high proportion of ovarian cancers are at an advanced stage when diagnosed.[1] The most lethal of these tumours is high-grade serous carcinoma, largely because it is invariably discovered after having spread to the adjacent peritoneal surfaces.[2] This aggressiveness of this tumour behavior is in stark contrast to other serous epithelial neoplasms, including serous tumours of borderline malignancy and low-grade serous carcinomas. Other epithelial malignancies in the ovary, such as endometrioid and mucinous tumours, each display their own spectrum of benign, borderline and malignant. Thus, the single term 'ovarian cancer' belies the complexity of origins and difficulties in management and prevention. At the core of this conundrum is an unanswered question that plagues those who study not only ovarian cancer but tumours in many other sites of the human body: which is the site of origin and the earliest event of tumorigenesis? In the realm of ovarian cancer, this problem has been addressed repeatedly.

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Some concepts are based on tangible evidence, whereas others are highly theoretical; however, their solutions are vital to our understanding of how these tumours develop and, quite possibly, to designing strategies for prevention and early detection.

The purpose of this review is to define the parameters needed to study the range of pelvic epithelial malignancies and then to discuss their subclassification. It will first focus on those tumours which have a developmental pathway in which pre-existing conditions can be ascertained, largely those tumours in the endometrioid, mucinous and low-grade serous tumour categories. It will then address high-grade serous tumours, whose origin and development from precursor lesions remain obscure. In this review, the terms 'ovarian (or tubal) cancer' will be used when the origin is presumed to be in one of these sites, and 'pelvic (serous) cancer' will generically include all tumours arising in the pelvic region.

TUMOUR ORIGINS

This discussion will focus on the distal fallopian tube and the ovary as sites of tumour origin. Provided a source in the ovary or tube has been excluded, primary peritoneal malignancies are presumed to arise from sites similar to those in the ovary, being endosalpingiosis or endometriosis. When addressing origins in the ovary, Mullerian inclusions will be held synonymous with endosalpingiosis, simply because there is no reliable way to distinguish the two. Similarly, the derivation of endometriosis or endometriotic cysts, from the endometrium versus transformation of ovarian surface epithelium (OSE), will not be debated. The OSE is accepted to be a distinct cell population that embodies both mesothelial and epithelial characteristics, and from which Mullerian inclusions can be derived.[3]

DEFINING PARAMETERS OF THE PATHWAY

Location of susceptible epithelium

Excluding the endometrium, pelvic malignancies can be presumed to arise from three distinct sites: the ovary, including ovarian surface, the endosalpinx, and the peritoneal surface (Table 1). The easiest to appreciate is the endosalpinx, where direct transitions from benign to malignant can be witnessed, albeit uncommonly. Less well understood is the OSE, including Mullerian inclusions in the ovarian cortex, because early cancers are rarely identified in these sites. Most mysterious is the peritoneum, although Mullerian inclusions in the form of endosalpingiosis and endometriosis are often present. The factors that influence the emergence of Mullerian epithelium in the ovaries or on the peritoneum are poorly understood, but are ultimately vital to tumour development. It is important to emphasize that site of origin influences tumour type. The salpinx rarely gives rise to borderline or well-differentiated serous carcinomas, endometrioid carcinomas and mucinous tumors, implying that the ovarian inclusion cyst retains a specific capability to give rise to these tumours. The fact that most tumours arise within the ovary suggests further that the ovarian cortex is involved in this process.

Target cell phenotype

There is increasing evidence that target cell type plays a role in tumorigenesis; however, in the ovary it may at times be impossible to tease out the molecular pathways from cell differentiation because they may both be tightly linked.[4] At least four types of epithelium come into play, including the OSE, often referred to as a simple or uncommitted epithelium, Mullerian inclusion cysts (MICs), which resemble salpingeal epithelium, endometriosis, and the salpinx. Specific molecular events that initiate neoplasia may be more or less restricted to certain cell types, but whether this is a function of the cells or their location (ovary versus tube) is not clear.[5,6] In the fallopian tube, epithelial malignancies are primarily, but not exclusively, assigned to the secretory cells. Borderline tumours of the ovary can exhibit a blend of both secretory and ciliated differentiation. Whether these differences reflect inherently different cell targets, environment-specific genotoxic stresses, or both, is difficult to ascertain. Phenotypic plasticity comes into play with tumour phenotypes that have no benign counterpart in the ovary, such as mucinous tumours. An attractive hypothesis proposes that coordinated expression of homeobox genes governs the different Mullerian cell phenotypes, suggesting that the transition from serous inclusion cyst to mucinous cystadenoma involves a switching on of HOXA11.[7] The orderly expression of members of the *HOX* gene family governs Mullerian differentiation during development and resurfaces in their associated cell types in malignancies. Moreover, differential expression of these genes *in vitro* in transformed mouse OSE generates neoplasms that recapitulate their benign phenotypes: HOX 9, 10 and 11 leading to high-grade serous, endometrioid and mucinous tumours, respectively.[7] Naturally, this model best fits a scenario in which uncommitted OSE provides the obligate progenitor cell to these three types of ovarian cancer. Alternatively, these genes could be switched on to mediate the transition from OSE to salpingeal-like

Table 1. Summary of parameters involved in pelvic epithelial tumorigenesis[8,9,13,15,17,21,24,26,27,31,32,34]

Tumour	Site	Source	Cell target	Genetics	Benign precursor	Typical distribution and behaviour
IMucinous	Ovary	MIC	Mullerian*	HOXA11 K-ras BRAF C-Myc	Cystadenoma Adenofibroma	Unilateral, intracystic, localized
MMucinous	Ovary	Endometriosis MIC	Emoid	Unclear	Endometrioma Borderline	Uni or bilateral, intracystic, localized
Endometrioid	Ovary	Endometriosis MIC	Emoid	HOXA10 PTEN BRAF β-catenin C-Myc	Endometrioma Adenofibroma	Uni or bilateral, intracystic, localized, distant spread
Clear cell	Ovary	Endometriosis MIC	Emoid	Diverse C-Myc	Endometrioma Adenofibroma	Unilateral, localized, distant spread
LG serous	Ovary	MIC	Mullerian*	K-ras	Cystadenoma Adenofibroma	Bilateral, intracystic or surface, peritoneal spread
HG serous	Fimbria	Endosalpinx	Secretory cell	p53	p53 signature	Unilateral, intraepithelial, minimal invasion, serosal spread
HG serous	Ovary	MIC Endometriosis	Secretory Mullerian cell	p53 C-Myc	Endometrioma Cystadenoma (uncommon)	Dominant mass, bilateral, surface, peritoneal spread
HG serous	Ovary	OSE	OSE or Mullerian	p53	? p53 signature	Bilateral surface involvement peritoneal spread

MIC, Mullerian inclusion cyst; Emoid, Mullerian epithelium with endometrioid differentiation; IMucinous, Intestinal type mucinous tumours; MMucinous, Mullerian mucinous tumours.
*'Generic' Mullerian epithelium composed of ciliated and secretory cells.

Mullerian inclusions, endometriosis and, occasionally, mucinous epithelium.[7,8]

Genotoxic injury and initial gene alterations

The initial events preceding malignancy in the upper female genital tract are poorly understood. The target epithelium, whether it is on the ovarian surface or the fimbrial mucosa, must be exposed to a genotoxic insult, whether it is the result of ovulatory events, hormonal fluctuations, carcinogen exposure or spontaneous mutations rates associated with cell turnover. Three genetic disturbances that characterize mucinous and low-grade serous, endometrioid and high-grade serous neoplasms occur in the *K-ras*, *phosphatase and tensin homologue* (*PTEN*) and *p53* genes, respectively.[9,10] The mechanisms by which these events occur are not understood, but their tumour type specificity indicates a dependence on the target cell. All are common in tumours in humans. *K-ras* mutations are common and early in mucinous and low-grade serous tumours, but not in high-grade serous tumours. In the endometrium, *PTEN* mutations are common in normal epithelium, possibly reflecting an inevitable event in cells with a high turnover.[11] Mutations in *p53*, although frequently associated with advanced ovarian carcinomas, namely high-grade serous carcinomas, are also seen in non-neoplastic epithelium, including sun-exposed squamous epithelium and otherwise benign appearing salpingeal epithelium.[12] The latter will be discussed in greater detail below.

Risk factors for progression

The dominant known genetic risk factor for serous ovarian cancer is a heritable heterozygous *breast cancer gene* (*BRCA*) mutation, which increases the lifetime risk of ovarian cancer to >50% for BRCA1+ individuals.

This is in contrast to a risk of 1.3% in the general population. Approximately 15% of ovarian cancers are attributed to *BRCA* mutations. Exactly where in the pathway to pelvic cancer the BRCA haploinsufficiency plays its role is not clear. There is no consistent evidence that the normal fallopian tubes or ovaries from BRCA+ women differ in their appearance from randomly selected women without a family history of ovarian cancer. Preliminary evidence also indicates that the prevalence of recently proposed early or latent precursors (such as a p53 signature) are similar in both BRCA+ women and controls.[12] Other risk factors such as faulty DNA mismatch repair (hereditary non-polyposis colorectal cancer) are linked to a small percentage of ovarian endometrioid adenocarcinomas.

Microenvironment and tumour properties supporting growth and expansion

Once a malignancy develops, its biological behaviour may be further influenced by its location, inasmuch as the latter may vary in its support of tumour growth. Many large ovarian and peritoneal carcinomas are associated with a non-invasive or minimally invasive carcinoma of the distal fallopian tube, and the two have been demonstrated to contain the same *p53* mutations, signifying a common origin.[13] Although it would seem counterintuitive to assign the primary site to the fimbria in such cases, it could be explained by both the host tissue microenvironment and the capacity of this malignancy to propagate on peritoneal and ovarian surfaces and not on the endosalpinx. Both are well-known targets for metastatic carcinoma as opposed to the endosalpinx. Disparities in supporting environments would both influence initial tumour growth and, ultimately, the perception of its origin, particularly if the distant sites harboured larger tumour growth. BRCA+ serous cancers presenting in symptomatic women are classified as ovarian in >90%. In contrast, up to 100% of cancers discovered incidentally at the time of risk-reducing salpingo-oophorectomy are classified as tubal.[14] The most plausible explanation is that early malignancies in BRCA+ women most commonly arise in the tube but spread (and grow) preferentially at remote sites, resulting in misclassification as peritoneal and ovarian primaries. In stark contrast, mucinous carcinomas arise in the ovaries and are less likely to spread, having neither a high propensity to vascular space invasion or spread to serosal surfaces.

Perspectives on intervention or prevention

The range of intervention options varies as a function of the tumour type. Mucinous tumours and low-grade endometrioid adenocarcinomas are typically stage I (or II) when diagnosed and thus have an optimistic prognosis. Successful intervention in this group of tumours may be achieved by early detection alone, using techniques designed to detect an enlarged ovary. In contrast, interrupting the course of a pelvic serous carcinoma is virtually impossible with physical examination or ultrasound, simply because these tumours are more likely to have spread to the peritoneal surfaces at the time of detection. In this subset of tumours, successful intervention would require preventing the onset of malignancy by interrupting the development of a precursor lesion. Analogous scenarios play out in the cervix and uterus. Human papillomavirus vaccines prevent the earliest precursor lesions from occurring in the cervix and thus prevent cancer. Endometrioid endometrial cancers are often preceded by precursors (endometrial intraepithelial neoplasia) that can be identified and removed prior to cancer development. In contrast, uterine serous carcinomas, even in their earliest phases, appear at risk for distant spread, necessitating a strategy that would either identify or interrupt the earlier events in serous carcinogenesis.[15]

THE ENDOMETRIOID/CLEAR CELL SEQUENCE

Frequency and location

Endometrioid and clear cell carcinomas comprise about 25% of ovarian cancers (Figure 1). Approximately 75% of pelvic malignancies classified as endometrioid, Mullerian mucinous or clear cell arise from the ovary, and many of these stage I tumours arise in endometriosis.[16,17] Extraovarian tumours in the peritoneum, cul-de-sac and other sites are less common, but typically are associated with endometriosis.

Cell of origin

Pre-existing endometriosis is associated with approximately one-half of endometrioid adenocarcinomas and presumably is the source of these tumours. Alternatively, direct transformation of the OSE or Mullerian inclusions into endometrioid epithelium would explain those that are not. Endometrial carcinomas are occasionally reported in endosalpingeal epithelium, but are much less common.

Initial genetic events leading to endometriosis

There is evidence that endometriosis is monoclonal, but the mechanisms of endometriosis remain obscure.[18] A meta-analysis of polymorphisms has not revealed a consistent link with endometriosis.[19] Specific gene alterations have been identified, but their role is unknown.[20] Interestingly, aberrant methylation of

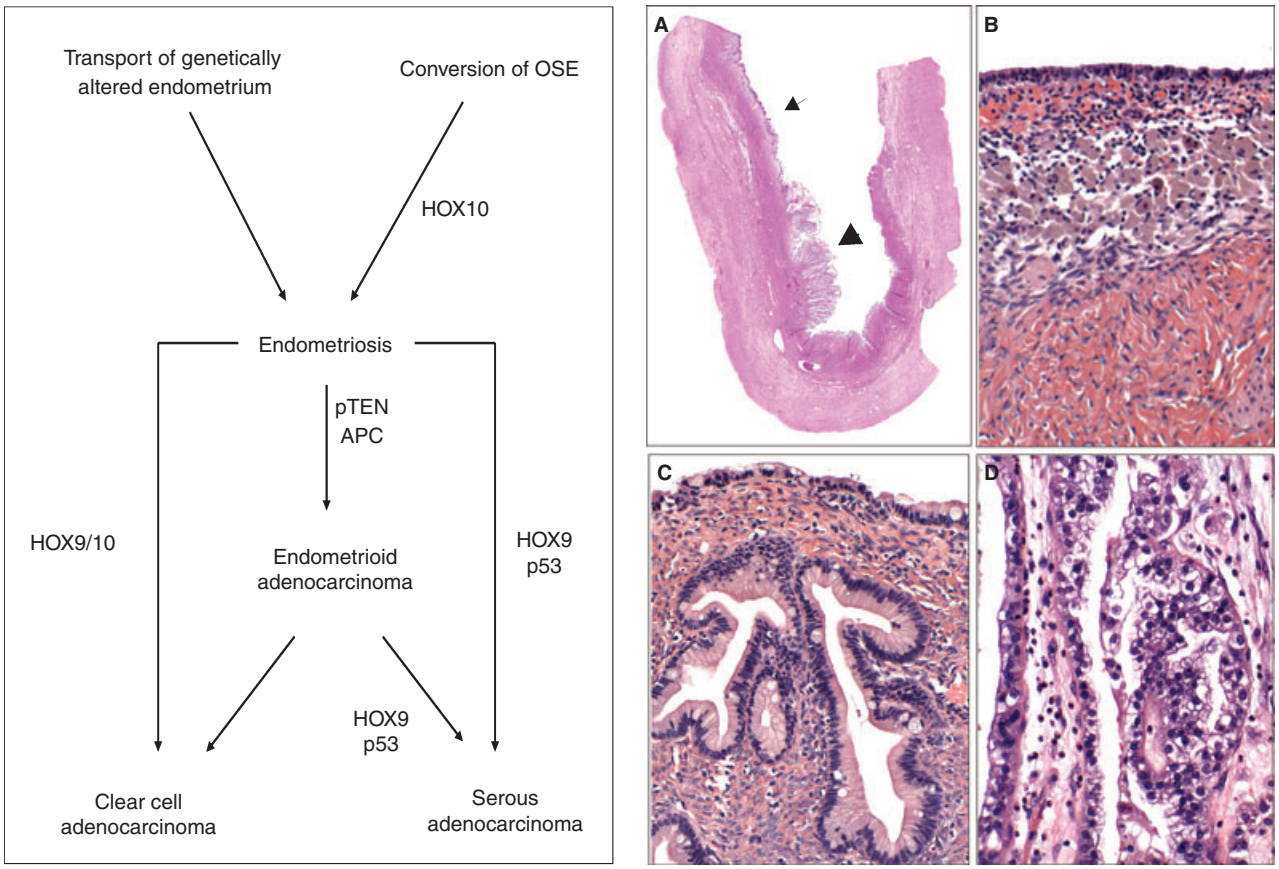

Figure 1. Schematic of the ovarian endometrioid carcinogenic pathway (left). Right, ovarian endometriotic cyst containing an endometrioid carcinoma (**A**), higher power of a focus of endometriotic cyst lining (**B**), mucinous metaplasia in the carcinoma (**C**), and focal clear cell carcinoma in the same tumour (**D**).

HOXA10 has been reported in endometriosis, sharing identical properties with eutopic endometrium from the same individual.[21] Based on this, some have cautiously proposed the endometrium as a source for these lesions.[22] Whether or not the shared molecular aberrations signify a causal relationship, they support other data indicating that endometriosis is biologically distinct from normal endometrium. Recently, common allelic loss patterns have been identified in both ovarian endometriosis and co-existing carcinoma.[23] Alterations in the chromosomal locus containing the *PTEN* gene have also been found in endometriosis and increase in frequency in adenocarcinomas.[24,25] Dinulescu *et al.* have described a mouse model for endometrioid carcinogenesis in which introduction of an oncogenic K-ras allele in the uterine horns led to extrauterine endometriosis.[26] A superimposed inactivating *PTEN* mutation was associated with the onset of endometrioid adenocarcinoma. However, although a similar pathway may be involved in the human, oncogenic *K-ras* mutations have not been identified, and the precise relationship between endometriosis and cancer risk, which is approximately 1%, is unresolved.[27,28]

Factors promoting progression to malignancy

Molecular changes linked to the progression from endometriosis to cancer include loss of PTEN expression, DNA mismatch repair and disturbances in other pathways.[29] Wu *et al.* have demonstrated further that defects in the Wnt/β-catenin and PI3K/PTEN pathways are associated with human endometrioid carcinomas. The authors divided endometrioid tumours into two groups: (i) grade 2 or 3 tumours harbouring *p53* mutations without disruption of Wnt/β-catenin and PI3K/PTEN pathways, and (ii) grade 1 tumours with mutations in the Wnt/β-catenin and PI3K/PTEN pathways, but without mutations in *p53*. *PTEN* mutations were concurrent with mutant *β-catenin*, but not mutant *K-ras*. In this study, *K-ras* mutation was relatively infrequent (7%), but it may represent an additional force driving carcinogenesis specifically in a subset of tumours with *p53* mutations. The study also

included a mouse model in which Apc and PTEN were conditionally inactivated in the OSE; these genetic changes produced endometrioid carcinomas with similar morphology to human disease.[30] In addition to endometrioid carcinomas, both serous and clear cell malignancies can arise in endometrial tissue.[31] *p53* mutations occur in some clear cell carcinomas, presumably acquired when malignancy develops. However, they are not consistently linked to clear cell carcinomas, which share genetic characteristics with clear cell carcinomas in other sites such as the kidney.[32,33] One feature found frequently in many endometrioid, clear cell and serous carcinomas is amplification of c-myc. However, amplification of this gene does not appear to be specific to a given cell type.[34,35]

Microenvironment

Most endometrioid carcinomas are discovered when confined to one or both ovaries, with or without co-existing endometrial involvement in 10–20%. Many originate within endometriotic cysts and do not involve the ovarian surface. A minority spread to the pelvic surfaces; most distant spread is to lymph nodes or distant sites, such as the lung. Overall, the outcome is improved relative to serous carcinomas because of the tendency for these tumors to be not only confined to the ovary when diagnosed, but also well differentiated.

Prevention

Prevention of endometriosis-related malignancies naturally would rest with the prevention of endometriosis, which is under investigation.[36]

THE MUCINOUS CARCINOGENESIS SEQUENCE

Frequency and location

Mucinous carcinomas comprise approximately 10% of ovarian cancers (Figure 2). Based on the relative rarity of mucinous tumours in extraovarian sites, it can be

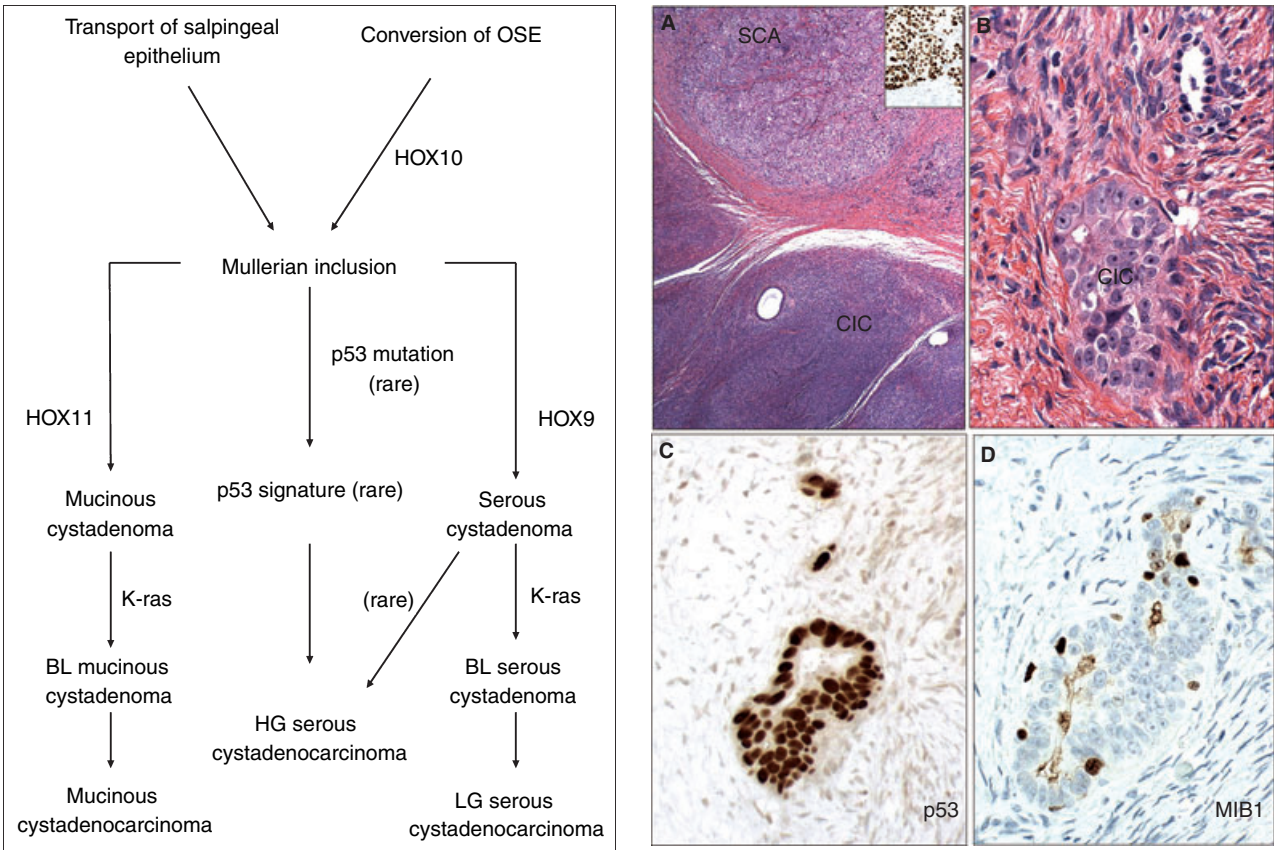

Figure 2. Schematic of pathways to mucinous, high- and low-grade serous tumours arising from the ovarian surface epithelium via Mullerian inclusions (left). Right, a serous carcinoma adherent to the ovarian surface is p53+ (inset). The ovarian cortex contains cortical Mullerian inclusions (CIC; **A**); a CIC in an adjacent field (**B**) is strongly immunopositive for p53 (**C**) and demonstrates a low MIB1 index (**D**) consistent with a p53 signature. Such changes have been proposed as precursors of ovarian serous carcinoma arising in CICs (J. Boyd, unpublished).

assumed that the precursor to these tumours lies in the ovary. Extraovarian mucinous tumours are rare, in the absence of a gastrointestinal source.

Cell of origin

The presumed source of mucinous tumours is Mullerian epithelium within the ovarian cortex that undergoes mucinous metaplasia in concert with HOXA11 expression. The expression profile of the intestinal mucinous lesions is consistent with the intestinal phenotype.[37] Mullerian mucinous tumours have a mixed epithelial phenotype, including ciliated, endometrioid and endocervical-like mucinous differentiation.[38,39] These tumours are often associated with endometriotic cysts, possibly manifesting as mucinous metaplasia of pre-existing endometrioid epithelium, similar to that seen in the endometrium.[40] Alternatively, these tumours could arise from cortical inclusion cysts via differential *HOXA* gene expression.[7]

Initial genetic events

At least two events are involved in the process of mucinous tumour development. The first is a shift in differentiation to a mucinous phenotype, which has been linked to *HOXA11* gene expression.[7] The second is a mutation in an oncogene. The most frequent genetic perturbation associated with mucinous neoplasms is the *K-ras* mutation.[41,42] In a large study of 95 mucinous tumours, *K-ras* mutations at codons 12/13 were detected in 68%, with mutations in codon 12 present in most, including 56%, 73% and 85% of mucinous cystadenomas, borderline mucinous tumours and carcinomas, respectively.[42] The broad association of *K-ras* mutations in these tumours and consistency of mutation locus in different regions of the same tumour underscore the *K-ras* mutation as an early event in ovarian mucinous carcinogenesis.[42]

Factors promoting progression

There are no known risk factors that distinguish benign intestinal-type mucinous tumours from carcinomas; both frequently co-exist as a continuum in the same ovarian specimen. As would be expected, other molecular events, such as promoter methylation, correlate with progression but have not been characterized in detail.[43]

Microenvironment

Mucinous tumours arise in the ovarian cortex, are typically unilateral, and tend to remain localized and intracystic. These tumours tend to displace the stroma by expansile rather than infiltrative growth. Excluding metastatic mucinous tumours, survival with mucinous carcinoma is the highest out of all epithelial ovarian malignancies.

Intervention

Prevention of death due to primary ovarian mucinous neoplasms is predicated on early detection and is aided substantially by the natural history of this tumour, which is often found at an early stage.

THE SEROUS CARCINOGENIC SEQUENCE

Site of origin

Serous tumours have traditionally been assumed to arise from the ovary (Figures 2 and 3). Although the ovary is an undisputed source of many high-grade malignancies, the precise percentage of serous carcinomas arising in this pathway has not been ascertained.[44,45] This is a function of both the difficulty in pinpointing the origin in advanced tumours and the fact that the fallopian tube is emerging as an important site of origin.

The conundrum posed in attempts to pinpoint the origins of high-grade pelvic serous carcinomas is well illustrated by comparing the primary sites of women with *BRCA* mutations (BRCA+). Piek *et al.* have assigned the primary site of the neoplasm of symptomatic BRCA+ women to the ovary in >90% of cases. In contrast, smaller prospective studies of women undergoing risk-reduction salpingo-oophorectomy have demonstrated a source in the distal fallopian tube in up to 100%.[14,46] A recent study of consecutive women with pelvic serous cancer has suggested a source in the fallopian tube in approximately one-half.[13] These studies, although among the first of their type, indicate a need for careful investigation of the distal fallopian tube in all cases of pelvic serous cancer. We have recently analysed two variables that frequently imply site of origin, including endosalpingeal involvement (suggesting tubal origin) and dominant ovarian mass with one ovary at least three times larger than the other (suggesting the primary site of origin). Endosalpingeal involvement was seen in 21% of cases with a dominant ovarian mass in contrast to 79% of cases without a dominant ovarian mass (M. Roh and C. Crum, unpublished data). The impression from these observations was that some tumours arise in the ovary (dominant mass without endosalpinx involvement), others arise in the fallopian tube (equal involvement of both ovaries and endosalpinx) and still others may arise from the ovarian surface or another source close to or on the ovarian surface (equal involvement of both ovaries without endosalpingeal involvement). Together, these

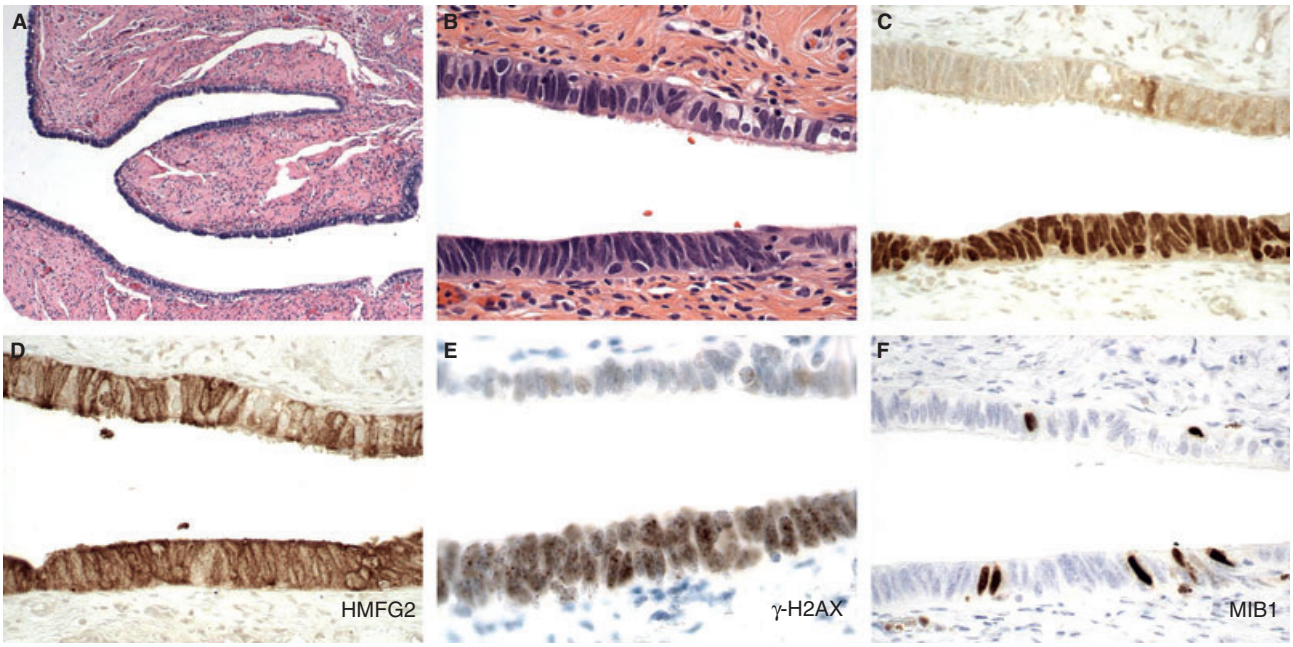

Figure 3. Histopathology and immunohistochemistry of p53 signatures in the distal fallopian tube, a proposed precursor of pelvic serous carcinoma. p53 signatures are most commonly detected in the fimbria (**A**), are inconspicuous on routine H&E immunohistochemistry (**B**), but are strongly immunopositive for p53 (**C**). The involved epithelium consists entirely of secretory cells as evidenced by homogeneous staining for HMFG2 (**D**). Like serous carcinomas, p53 signatures exhibit punctate immunopositivity for γ-H2AX (**E**); however, they exhibit no or only slightly increased proliferative activity relative to the normal mucosa (**F**).

finding indicate a multiplicity of sites of origin for pelvic serous cancer.

Cell of origin

Four cell populations are candidate targets for pelvic serous carcinogenesis and include OSE, Mullerian inclusions in the ovarian cortex, endometriosis and the endosalpinx. The most heavily studied up to now has been the OSE, which contains a primitive epithelium exhibiting features of both mesothelium and epithelium, while remaining distinct from the epithelium of the salpinx and cortical inclusions. Theoretically, this population can transit to serous carcinoma upon neoplastic transformation.[3] Alternatively, serous carcinoma emerges from OSE-derived Mullerian inclusions, which closely resemble the endosalpingeal epithelium.[5] Both are composed chiefly of ciliated and secretory cells.[47] These two cell types vary in their frequency in the endosalpinx as a function of hormonal stimuli and presumably are derived from the same progenitor cell. Evidence for this is as follows: (i) both phenotypes can be induced in cell culture from a common cell population;[48] (ii) proliferative activity is unique to the secretory cell type, implying that this population is the replicating cell population in the tube (M. Chang, E. Agoston and C. P. Crum, unpublished data); and (iii) low-grade serous tumours exhibit both secretory and ciliated cell differentiation (M. M. Parast, C. P. Crum and M. S. Hirsch, unpublished data).

The last observation underscores an important difference between low- and high-grade serous tumours that is most easily explained by the differentiation capabilities of the transformed cell. This will be discussed in greater detail below, but essentially, low-grade serous tumours retain the ability to undergo ciliated cell differentiation, whereas high-grade tumors do not. This may be a function of the fact that the factors influencing the transition from inclusion cyst to low-grade serous neoplasia are unique to the ovarian cortex.

Initial genetic events

A discussion of the early genetic events involved in serous carcinogenesis of both low- and high-grade serous tumours requires an appreciation of location of the target cell types and the molecular changes involved. Three fundamental differences between low- and high-grade serous tumours underscore the interdependency of these factors. Borderline and low-grade serous malignancies arise in the ovary in the great majority of cases, harbour *K-ras* mutations or mutations other than *p53*, and the cell population exhibits

a combination of secretory and ciliated differentiation. The presumed precursor is the MIC in the ovary via a benign serous cystadenoma. In contrast, the earliest universally recognized neoplastic change in high-grade serous carcinoma – tubal intraepithelial carcinoma – exhibits a non-ciliated (secretory cell or undifferentiated) phenotype. The most visible candidate precursor to this process is a clonal expansion of p53+ epithelium in the endosalpinx that is strictly secretory, implying that at this early stage, the epithelial type is fixed and unable to differentiate. This process, called the 'p53 signature', shares several attributes with tubal intraepithelial carcinoma, including: (i) fimbrial location in >80%; (ii) intense p53 immunoreactivity; (iii) involvement of the secretory cell; (iv) evidence of DNA damage as manifested by punctate immunopositivity for γ-H2AX;[49] (v) *p53* mutations in approximately 60%, and (vi) occasionally, direct continuity with malignant epithelium (tubal intraepithelial carcinoma; Table 2).[12] Similar lesions have been described occasionally in ovarian MICs, usually in association with serous carcinoma. In our experience, p53 signatures are very rare in MICs of BRCA+ women (A. K. Folkins and C. P. Crum, unpublished data). Occasionally, p53 signatures can be demonstrated in the ovarian cortex in the region of serous carcinomas (Figure 2).

Table 2. Data supporting the distal fallopian tube as a source of serous carcinoma

1. Similarity in expression profile between ovarian serous carcinomas and fallopian tube epithelium
2. Existence of tubal carcinomas in animal models (Hens) at high risk for ovarian cancer
3. Detection of early tubal carcinoma in over 70% of malignancies detected in BRCA+ women following risk-reducing salpingo-oophorectomy
4. Detection of early tubal carcinoma in one-half of ovarian and primary peritoneal serous carcinomas, irrespective of family history
5. Confirmed metastatic potential of non-invasive carcinomas of the distal fallopian tube
6. Identical *p53* mutations in tumours of the distal tube and remote ovarian and peritoneal serous carcinomas
7. Presence of a putative precursor (p53 signatures) in the distal tube that shares characteristics with tubal carcinomas including evidence of DNA damage, *p53* mutations, secretory cell specificity and fimbrial location
8. Rarity of p53 signatures in the normal ovarian cortex from BRCA+ women
9. Continuity between p53 signatures and early tubal carcinomas, supporting a transition from one to the other

A recent report has described alterations similar to p53 signatures in the OSE, a finding that supports the possible participation of this epithelium in serous carcinogenesis.

Factors promoting a transition from precursor to malignancy

The p53 signature is viewed as the initiation of a serous carcinogenic sequence in the distal fallopian tube (Figure 4).[50] It is found with nearly equal frequency (about one-third) in women with and without *BRCA* mutations, suggesting that it is not directly linked to a genetic risk factor (A. K. Folkins and C. P. Crum, unpublished data). It is presumed to undergo transition rarely to malignancy in women who do not have a *BRCA* mutation, consistent with its role as a precursor, which, by definition, requires an additional event to rarely undergo this transition. In the BRCA+ population, the *BRCA* mutation serves as a 'promotor' and the risk of developing a pelvic epithelial carcinoma in the BRCA+ patient is as high as 60% (BRCA1). Approximately 5% of risk-reducing prophylactic salpingo-oophorectomies in these women contain an early malignancy in the form of a tubal intraepithelial carcinoma. In some, we have observed multiple p53+ epithelial lesions ranging from p53 signatures to intraepithelial carcinomas in the same fallopian tube. Although we would still not exclude a role of BRCA status in the development of p53 signatures, our impression from this observation is that BRCA status may also influence the transition from p53 signature to intraepithelial carcinoma. This would be consistent with the high frequency of p53 signatures in all women and the higher risk of serous cancer in the BRCA population. Several scenarios could explain the role of BRCA status in lesion progression in the fallopian tube. In one, haploinsufficiency of BRCA would undermine the normal response to DNA damage or control of the cell cycle. In another, loss of the second allele would influence the transition from signature to malignancy.

Microenvironment and tumour spread

The most striking feature of serous malignancies that impacts on their prognosis is their propensity to spread to the peritoneal surfaces. This is a function of their capacity to grow on the peritoneum combined with their origin in sites (fimbria or ovarian surface) open to the peritoneal cavity. A study comparing genetic makeup between components of the same ovarian tumour at different sites has revealed differences in

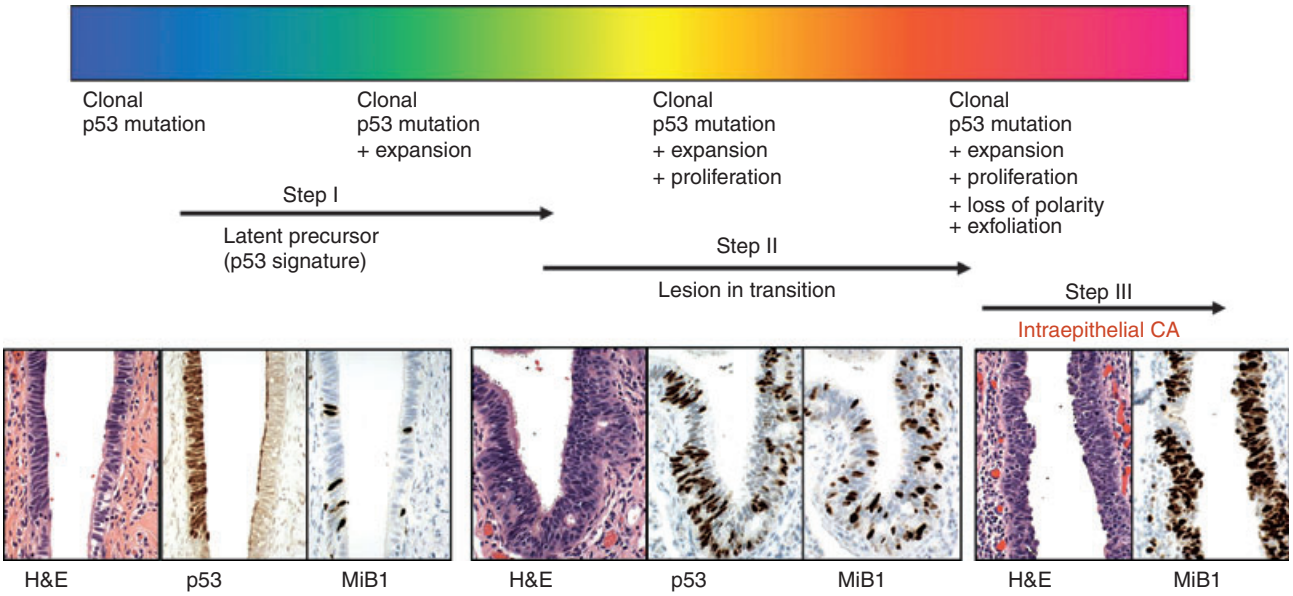

Figure 4. The serous carcinogenesis sequence in the fallopian tube consists of a spectrum of changes initiating with *p53* mutations (p53 signatures) and terminating in tubal intraepithelial carcinoma. In some instances, intermediate forms (lesions in transition) can be identified (from Jarboe *et al.*[49]).

allelic imbalance indicating that progressive genetic alterations occur over time.[51] However, it remains to be determined whether specific genetic events are required for tumour spread. Small studies of antigen expression have not shown discernible differences.[52] Moreover, the proclivity of serous carcinomas for peritoneal (including ovarian) surfaces does not appear to be acquired in a step-wise fashion after tumour development, as evidenced by adverse outcomes associated with intraepithelial carcinomas of the tube or uterine surface. This is particularly important in terms of early prevention, as it is likely that the window of opportunity to interrupt spread once a malignancy has developed is so small that it precludes reducing mortality by programmes targeting early detection of this malignancy.

Intervention

Preventing serous malignancies that arise in the fallopian tube is currently based on removal of the entire adnexae, which is reserved for women at high risk for this disease, including BRCA+ individuals. Preliminary evidence suggests that p53 signatures may share some of the same risk factors as ovarian cancer, leaving open whether oral contraceptives and tubal ligation would influence their development (A. K. Folkins and C. P. Crum, unpublished data). Presuming that p53 signatures are derived from ovulation-related oxidative injury to the distal fallopian tube, their development may be interrupted by therapies that reduced this likelihood. However, considerable research remains to be done to clarify the susceptible cell targets for this process and the factors that promote transition from p53 signature to serous malignancy.

Conclusion

The two-pathway concept of ovarian carcinogenesis has highlighted the distinct molecular differences between low- and high-grade serous tumours of the ovary. This review has introduced a series of variables that must be addressed to understand more completely the pathogenesis of these tumours, specifically their origins. While the origin of ovarian serous carcinomas remains multifactorial, possibly including endometriosis, MICs and OSE, the growing evidence implicating the fallopian tube as a potential site of origin merits its inclusion in the possible pathways. The recent description of the serous carcinogenic pathway in the distal tube explains a significant subset of these tumours and provides a visible entity that can be studied in greater detail. Although not all pelvic serous malignancies arise in the salpinx, the model constructed for this site may have applications to other sites and add to our knowledge of serous carcinogenesis and prevention.

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