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REVIEW ARTICLE

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Molecular pathology of ovarian carcinomas

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Abstract There is evidence that ovarian cancer may be derived from the progressive transformation of benign and/or borderline tumours. Mutations involving different oncogenes and tumour suppressor genes accumulate during the process of malignant transformation, and the alterations of genes involved in the pathogenesis of familial ovarian cancer are probably early events in ovarian tumorigenesis. *BRCA-1* and *BRCA-2* act as classical tumour suppressor genes in hereditary tumours, but their role in sporadic tumours remains controversial; however, a high frequency of allele losses in *BRCA-1* (17q) and *BRCA-2* (13q) loci has been observed in both familial and sporadic tumours. The possible role of mismatch repair genes and microsatellite instability is also controversial, but a role for them has been proposed in borderline tumours. Mutations in K-*ras* are specific for mucinous tumours and may be related to mucinous differentiation. Finally, a role in tumour progression has been proposed for both c-*erb* B-2 and *p53*, but their practical value in prognosis remains questionable.

Key words Ovary · Oncogenes · Tumour suppressor genes · Pathogenesis · Tumours

Introduction

The most popular hypothesis about the development of ovarian epithelial-stromal tumours is that they originate from small surface epithelial inclusion cysts [66]. It is believed that these cysts develop from invaginations of the ovarian surface epithelium and the underlying stroma as a result of repeated ovulations. Because the surface epithelium is derived from the coelomic epithelium, which gives rise to the müllerian ducts, it is accepted that the surface epithelium is capable of differentiating into serous (tubal), mucinous, endometrioid or transitional epithelium. In patients with epithelial tumours, the high incidence of hyperplasia and müllerian metaplasia of the surface epithelium or epithelial inclusion cysts of the contralateral ovary stresses the potential role of these changes as a substrate for the development of ovarian carcinomas [68]. The epithelial-stromal tumours that occur in this setting may be of three distinct histological and biological types: benign, borderline (low malignant potential) and malignant. There is evidence indicating that malignant epithelial-stromal tumours may sometimes result from the progressive transformation of benign and/or borderline tumours [67, 86]. The fact that ovarian carcinomas are often of mixed histological grades suggests that the higher grade components may have arisen from the low-grade elements by way of clonal expansion. In fact, histologically benign epithelium is found in 100% of both serous and mucinous borderline tumours, 90% of mucinous cystadenocarcinomas, and 56% of serous cystadenocarcinomas [67]. Moreover, it has been demonstrated that the histologically benign epithelium adjacent to ovarian cancers and the benign-looking areas of ovarian carcinomas frequently show the molecular alterations of the high-grade component [13, 100].

Cancer results from the accumulation of a number of different mutations involving oncogenes and tumour suppressor genes. Oncogenes are genes that encode proteins with important roles in cell growth and embryogenesis; however, when they are expressed in excessive amounts or in altered forms, they may induce uncontrolled cell proliferation. Tumour suppressor genes, formerly called anti-oncogenes, produce proteins involved in the negative control of cell growth. In contrast to oncogenes, both alleles of the tumour suppressor genes must be inactivated before the carcinogenic effect can take place. It is thought that in the ovary, as in other tumours, accumulation of genetic abnormalities may occur during the progressive transformation of benign and/or borderline tumours into carcinomas [66].

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There are many oncogenes and tumour suppressor genes that have been thought to be involved in the pathogenesis and progression of ovarian cancers. In this review the roles of several of them and their possible role in tumour development or progression will be discussed, together with their potential value for the management of patients with ovarian cancer. In the first part we will look at those genes that are probably involved in the pathogenesis of familial ovarian cancers; their dysfunction is thought to be an early event in ovarian tumorigenesis. Later, the role of genes involved in cell differentiation will be commented upon, with special emphasis on k-*ras* and its role in ovarian mucinous tumours. Finally, we will discuss the importance of genes that may be involved in tumour progression. The literature on this subject is both extensive and dispersed, and this review does not intend to be fully comprehensive; only the most significant changes are discussed here.

Hereditary ovarian cancers are infrequent. Three distinct variants have been recognized [3]: (a) site-specific ovarian cancer, in which only ovarian cancers occur (approximately 10–15% of all hereditary ovarian cancer cases) [3, 7]; (b) the breast-ovarian cancer syndrome, in which families show an excess of both breast and ovarian cancer development (65–75% of all cases) [3, 7]; and (c) ovarian cancer associated with the hereditary nonpolyposis colon cancer syndrome (Lynch syndrome type II [95], which accounts for 10–15% of cases) [3, 7].

It has been speculated that the genes responsible for the familial forms of ovarian cancer may also be involved in the more common sporadic tumours. However, it is important to take into account that there are several important differences between the familial and the sporadic forms of ovarian carcinoma. For example, borderline tumours and mucinous carcinomas are very rare in hereditary ovarian cancer families, while serous tumours account for the vast majority of the "familial" neoplasms [3, 58, 65, 72, 77]. It has been suggested that the sporadic serous tumours may share some genetic mechanisms with the familial tumours, while the molecular alterations involved in other tumour types may be significantly different [64].

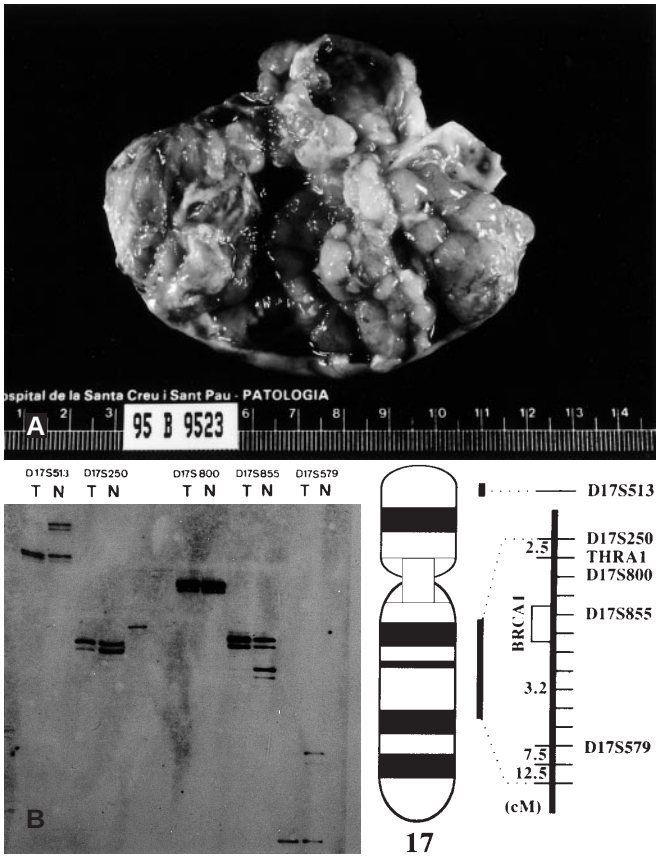

Fig. 1 **A** Ovarian carcinoma from a patient with history of bilateral breast cancer. The patient belongs to a family with hereditary breast-ovary cancer syndrome. **B** Demonstration of allele losses at several polymorphic chromosome 17 microsatellites in the ovarian tumour (*T* tumour DNA, *N* DNA obtained from peripheral blood)

BRCA-1 and *BRCA-2*

The two genes responsible for about two thirds of the cases of the familiar breast-ovarian cancer syndrome are *BRCA-1* and *BRCA-2*, which have been mapped to chromosomes 17q and 13q, respectively.

A wide array of germline mutations have been identified in the *BRCA-1* gene [8, 21, 53, 80], and most of them are detected in only one or two families [60]. An exception to this is a frame-shift mutation at position 185 in exon 2, which has been identified quite frequently in Ashkenazi Jewish families [56]. There is probably a wide range of neoplastic potential in each of these mutations; it has been suggested that the mutations involving the two terminal conserved domains of the gene (the amino and carboxyl terminals) may be associated with the development of highly proliferative breast carcinomas [85], although the same has not been shown for ovarian carcinomas. Germline mutations in *BRCA-1* are responsible for approximately 80% of hereditary ovarian cancers occurring within the context of the breast and ovarian cancer syndrome [7]. Ovarian cancer risk is increased in women with *BRCA-1* germline mutations when one or two rare alleles of the H-*ras* variable number of tandem repeats polymorphism are present [62].

In patients from families with the breast-ovarian cancer syndrome, the *BRCA-1* gene acts as a classical tumour suppressor gene; germline mutations are strongly associated with somatic allele losses involving the wild-type chromosome 17q, fulfilling Knudson's two-hit model for tumour suppressor genes [11] (Fig. 1). The fact that in 40–75% of sporadic cancers, somatic allele losses were also detected in 17q supports the hypothesis that in sporadic ovarian cancers *BRCA-1* might also function as a tumour suppressor gene [73, 74]. However, the frequency of *BRCA-1* somatic mutations in sporadic ovarian cancers is rather low [23, 31, 52, 88], and it has been suggested that *BRCA-1* may have a role in the development of hereditary cancers and not of sporadic tumours,

like WT1 in Wilms' tumour [93]. However, the high frequency of 17q allele losses in sporadic ovarian tumours suggest that some other genes on this chromosome may also contribute to the development of ovarian tumours [33, 40]. One study has shown that allele losses in 17q are more frequent in serous tumours than in endometrioid or mucinous neoplasms, suggesting that there might be some correlation between such genetic alterations and the histological type of the tumours [64]. A relationship between allele losses on chromosome 17 and advanced age has been reported recently [63].

The *BRCA-2* gene is also responsible for the hereditary breast-ovary cancer syndrome in several families [97]. The incidence of ovarian cancer compared with that of breast cancer appears to be much lower than in *BRCA-1*-linked families, although germline mutations of *BRCA-2* may be found in patients with late-onset ovarian cancers and without a remarkable family history with regard to breast or ovarian carcinoma [87]. It seems that inherited *BRCA-2* mutations contribute to ovarian cancer with a lower penetrance than *BRCA-1*, but that the number of ovarian cancers that result from them may be higher than previously estimated [7]. Like *BRCA-1*, somatic mutations of the *BRCA-2* gene have rarely been detected in sporadic ovarian cancers [19], although a high frequency of allele deletions occur in the 13q region [38].

Recently, the physiological role of *BRCA-1* and *BRCA-2* proteins has been elucidated. These proteins appear to be essential cofactors in the Rad 51-dependent DNA repair of double-strand breaks [78, 79].

Microsatellite instability

Ovarian cancer may occur in patients with the hereditary nonpolyposis colon cancer syndrome (HNPCC), which is characterized by an autosomal dominant inheritance of predominantly right-sided colonic cancer in the absence of colonic polyposis [3]. Recently, several groups have observed that HNPCC cells exhibit instability of microsatellite (MI) DNA sequences. Microsatellite DNA sequences are short tandem repeats made up of di- or trinucleotides that are distributed throughout the genome. The most common dinucleotide sequence in eukaryotes is the (CA)n repeat; and there are 50,000–100,000 (CA)n repeats in the entire human genome. The genes shown to be responsible for MI encode proteins involved in DNA mismatch repair (hMSH2, hMLH1, hPMS1 or hPMS2). Mutations of these genes alter the ability of the cells to repair errors produced during DNA replication [1, 32, 92]. Therefore, cells with mutated mismatch repair genes replicate DNA mistakes more frequently than normal cells.

Subsequently, MI has been also detected in sporadic cancers of different locations, such as colon [92], stomach and endometrium [36, 70]. Tumours exhibiting MI are thought to be replication error-positive (RER+). It should be emphasized that the diagnosis of MI should only be established when two or more loci are affected and at least five loci have been tested.

Indirect data suggest that MI may play a role in the genesis of ovarian cancer: family members of HNPCC sufferers have a 3.5-fold increase in the risk of ovarian cancer over that expected in the general population [3]; and MI is known to occur in endometrial carcinoma [36, 70], a tumour closely related to ovarian cancer. However, there is no general agreement about the frequency of MI in sporadic ovarian cancers. It appears to be very low in some series [42], but quite high in others [39]. A relationship between MI and some morphological features has been suggested in some series: for instance, MI has been specifically associated with ovarian endometrioid carcinomas and borderline tumours [89].

Other chromosomal abnormalities have also been associated with ovarian tumours. For example, allele losses have been detected in 6q (particularly at 6q27) [91], in several loci on chromosome 11 [24], and in chromosome 22 in several loci distal to the *NF2* gene [18]. Chromosomes 1, 3, 9, 14, 15, 18, 19 and X have also been implicated [4, 43, 91]. It has been demonstrated that widespread allele losses in all these chromosomes occur only occasionally in benign (8%) and borderline (11%) tumours, whereas they are found in up to 77% of carcinomas [75].

K-*ras*

Activated *ras* oncogenes have been detected in a wide variety of human tumours. The incidence, the *ras* gene implicated, and the type of mutation vary according to the histological type and the location of the neoplasm. In the ovary, K-*ras* mutations are far more common in mucinous than in nonmucinous epithelial-stromal tumours. Enamoto et al. first detected K-*ras* mutations in 6 out of 8 (75%) mucinous tumours in comparison with 3 out of 22 (14%) nonmucinous neoplasms, and the mutations were always detected at codon 12 [17]. It has since been confirmed that K-*ras* mutations are characteristic of ovarian mucinous tumours and distinguish them from

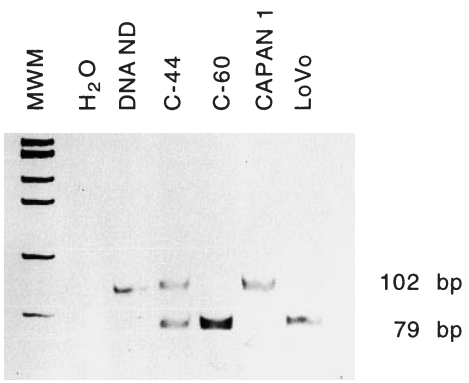

Fig. 2 Restriction fragment length polymorphism (RFLP-PCR) analysis reveals a point mutation at codon 12 of K-*ras* in a mucinous intestinal cystadenoma of the ovary (case 44). The 102- and 79-bp bands correspond to the mutant and normal alleles, respectively (*CAPAN-1* positive control)

other histological types; the mutations may be detected in benign, borderline and malignant tumours with increasing frequencies [13]. Moreover, because of the common coexistence of components with different degree of malignancy (benign, borderline, malignant) within ovarian mucinous tumours, and since it is accepted – at least in some cases – that the low-grade component can precede development of the high-grade tumour, the presence of identical mutations in both neoplastic components strongly supports the hypothesis that K-*ras* mutations are early events in the development of mucinous ovarian tumours [13] (Fig. 2).

Analysis of ovarian nonmucinous tumours has shown a low frequency of K-*ras* mutations, as well as a lack of prognostic significance of the mutations in malignant tumours [14]. Interestingly, among benign nonmucinous lesions, K-*ras* mutations are particularly common in Brenner tumours [14]. This is probably related to the fact that Brenner tumours often contain areas of mucinous differentiation, and it supports the hypothesis that K-*ras* mutations are closely related to the mucinous phenotype [14].

c-*erb* B-2 (Her2/neu)

The proto-oncogene c-*erb* B-2 codes for a cell surface glycoprotein that is homologous to the epidermal growth factor receptor (c-*erb* B-1). C-*erb* B-2 activation by amplification and/or overexpression is a common phenomenon in breast and ovarian carcinomas [2, 71, 84].

C-*erb* B-2 activation may be assessed by several different techniques. The number of copies of c-*erb* B-2 can be quantified by conventional Southern blot analysis, by differential PCR (Fig. 3), or even by interphase cytogenetics (FISH) [61]. Beyond this, we also may look for c-*erb* B-2 expression by Northern blot analysis, in situ hybridization (Fig. 4) or immunohistochemistry (Fig. 5). In breast carcinomas, it has been stated that if all these techniques were performed appropriately the discrepancies between them would be insignificant [57], but it has been suggested that in ovarian tumours, c-*erb* B2 amplification does not necesarily correlate with protein overexpression.

C-*erb* B-2 amplification and/or overexpression occur in 10–50% of ovarian carcinomas. Several studies have shown that c-*erb* B-2 activation is associated with a poor prognosis and lower survival rates [2]. It has also been suggested that c-*erb* B-2 overexpression may be a useful marker to identify the patients most likely to benefit from high-dose chemotherapy (T) [51]. However, in other series, similar results have not been obtained [26, 83], and the practical value of c-*erb* B-2 activation in ovarian tumours remains questionable.

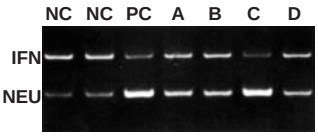

Fig. 3 Demonstration of c-*erb* B-2 (Her2/neu) amplification by differential PCR. Notice that bands corresponding to neu in the positive control (*PC*) and in *lane C* are more intense than those corresponding to interferon (*NC* negative control)

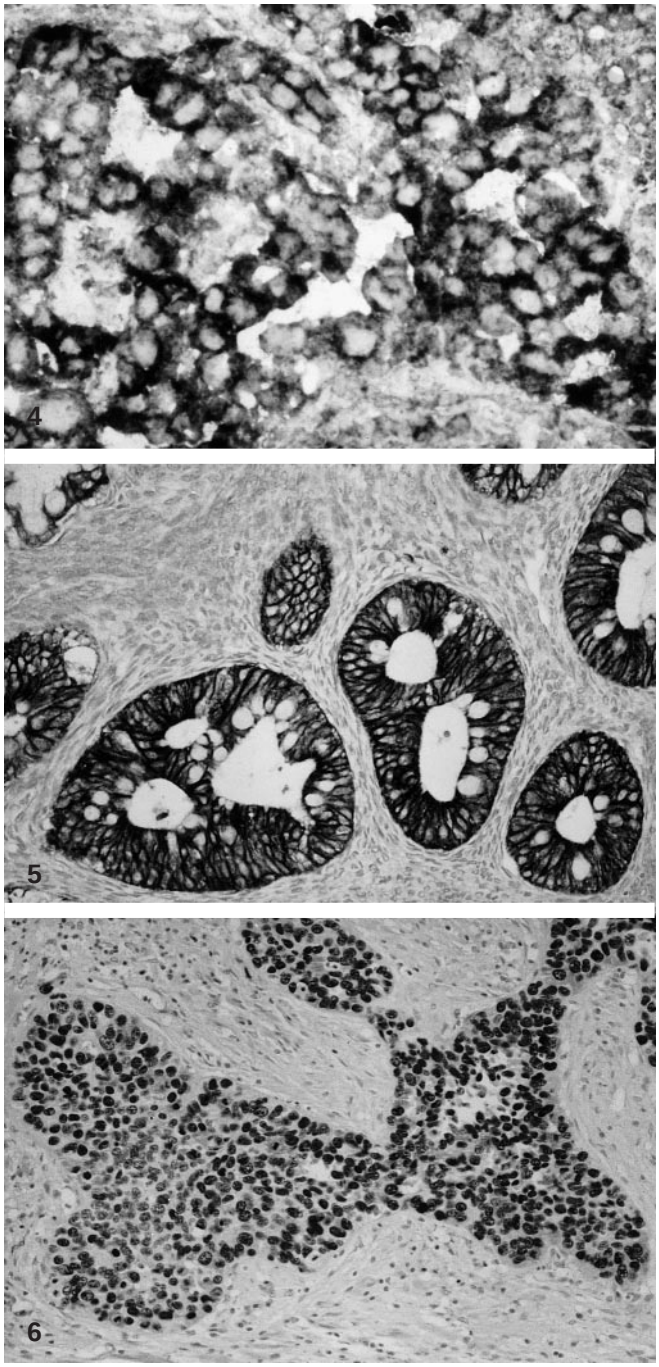

Fig. 4 C-*erb* B-2 overexpression in an ovarian carcinoma demonstrated by in situ hybridization with digoxigenin-labelled riboprobes

Fig. 5 Immunohistochemical expression of c-*erb* B-2 in an endometrioid ovarian carcinoma of the ovary

Fig. 6 Immunohistochemical expression of p53 in an undifferentiated ovarian carcinoma of the ovary

p53

The *p53* tumour-suppressor gene encompasses 16–20 kb of DNA in the short arm of the human chromosome 17 at position 17p13.1. This gene encodes a 393-aminoacid nuclear phosphoprotein involved in regulation of the cell division. *p53* is mutated in about 60% of human tumours. The mutation often results in prolongation of the half-life of the p53 product, so that the accumulated protein becomes detectable by immunohistochemistry (Fig. 6). Although a 96% concordance between positivity by immunohistochemistry and the presence of missense mutations has been reported in some series [55], false-negative and false-positive results can occur as a result of technical problems, abnormal p53 protein stabilization or alterations of the normal degradation process [9]. Moreover, nonsense mutations, splicing mutations and deletions do not usually result in *p53* immunoreaction.

Alterations of *p53* tumour-suppressor genes including allelic losses, mutation and overexpression are frequent in ovarian carcinomas [20, 50, 54]. Some investigators have suggested that *p53* abnormalities are involved in the development and progression of a significant proportion of ovarian carcinomas, especially in advanced stages [59], whereas others support the idea that *p53* gene alterations might be earlier genetic events [45]. It has also been postulated that *p53* immunoreactivity may have prognostic value in certain subsets of patients with ovarian cancer [6, 27, 41]. In fact, *p53* abnormalities have been found to correlate with advanced stage in several series. Nevertheless, it has also been suggested that in stage I tumours, immunostaining for *p53* may represent a negative prognostic factor and might allow the identification of a subset of aggressive neoplasms in which different therapeutic approaches would be applicable [26, 45]. However, a critical review of the literature shows that the clinical significance of *p53* overexpression or mutation in patients with ovarian cancer remains uncertain; the vast majority of studies demonstrate that *p53* alterations may be associated with poor survival in univariate but not in multivariate analysis [16].

It is important to note that *p53* mutations are rather infrequent in borderline tumours [46, 96]; two recent reports have shown that *p53* expression in borderline lesions may be associated with atypia, increased mitotic activity, microinvasion and the presence of coexistent carcinoma [41, 44]. Such results support the theory that *p53* could contribute to the malignant transformation. A recent study has shown that *p53* abnormalities are quite frequent in the so-called micropapillary serous carcinoma, a type of tumour that shares pathological features with both serous borderline and malignant ovarian tumours. p53 immunoreactivity was moderately intense and diffusely distributed in micropapillary serous carcinoma, whereas it was weak and focal in borderline tumours, and very intense and diffuse in serous carcinoma [35]. However, neither borderline nor micropapillary serous carcinomas exhibited *p53* mutations while they were present in all immunopositive serous carcinomas.

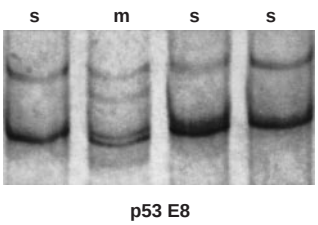

Fig. 7 Analysis of p53 mutations by single-strand conformation polymorphism (*SSCP-PCR*). Note the presence of abnormal bands in *lane m* in comparison with wild type p53 (*lane S*)

Finally, the role of *p53* in DNA repair and apoptosis has led to the hypothesis that *p53* mutations may determine resistance to the anticancer drugs that induce apoptosis in tumour cells (see below).

Cell cycle regulators

Cell cycle progression is controlled by protein complexes composed of cyclins and cyclin-dependent kinases (Cdk). Five major classes of mammalian cyclins (A, B, C, D, E) have been characterized. Cyclins C, D1–3, and E regulate the transition from G1 to S phase, whereas cyclins A and B1-2 are regulators of the transition to mitosis. Multiple Cdk molecules are being described and their cyclin partners and patterns of cell cycle specificity identified [10]. Specific heterodimers composed of a cyclin and a Cdk exert their regulatory function on several proteins involved in cell cycle transitions, such as retinoblastoma (RB) and p53. Moreover, an additional group of cell cycle regulators, designated as Cdk inhibitory molecules (CKI) has been identified. *p21/WAF1*, *p16/INK4A*, *p15/INK4B*, *p27/Kip1*, *p57*, *p19*, and *p18* are members of this family of genes [10]. It has been postulated that the aberrant expression of all these genes would deregulate cell growth and therefore have a major impact on tumour development or progression [10]. In fact, mutations and overexpression of cyclins and Cdk transform them into oncogenes, whereas CKI are regarded as tumour suppressor genes [10]. The possible role of cell cycle regulators in ovarian tumorigenesis, however, has not been fully elucidated and discrepant results have been obtained [5]. A recent study has demonstrated cyclin D1 overexpression in 26% of ovarian tumours, most frequently associated with borderline and well-differentiated carcinomas [98], while a previous one showed cyclin D1 overexpression in 18%, cyclin D1 amplification in 3.3%, and cyclin E amplification in 12.5% of the tumours [12]. Moreover, a number of studies have analyzed the *p16/INK4A* gene in ovarian tumours; in one of these, *p16* deletions, mutations or suppressed transcription were detected in 16.7% primary ovarian cancers and 45% of ovarian cancer cell lines [34]. A second study demonstrated reduced expression of *p16* in 22 out of 60 tumours [22], while, in contrast, a third group of investigators detected *p16* overexpression in 87% of the tumours [82].

There is general agreement that the *Rb-1* gene probably does not have a significant role in ovarian tumorigenesis; although ovarian cancers frequently show deletions

in the region of 13q that contains *Rb-1*, the tumours usually retain a functional copy of the gene, suggesting the existence of a different tumour suppressor gene in this region [15, 38, 47, 48, 76].

Apoptosis

There is now an extensive body of literature indicating that apoptosis can provide useful prognostic information in ovarian cancer [49]. Several studies have indicated that the apoptotic index (the percentage of apoptotic cells detected by terminal deoxynucleotidyltransferase-mediated deoxyuridine triphosphate-biotin nick end labelling, TUNEL) correlates with mitotic and Ki-67 indices, high histological grade and short overall survival [99].

Apoptosis is modulated by a large number of proto-oncogenes and tumour suppressor genes, including *bcl-2*, *c-myc*, and *p53*. Interestingly *bcl-2* expression shows inverse relationships with the apoptotic index in ovarian carcinomas, and, as in tumours of other sites, with p53 expression, high histological grade and survival [28]. *c-myc* is amplified in 20–30% of ovarian cancers, but no definite correlation with prognosis has been found [90]. The p53 protein induces growth arrest or apoptosis following DNA damage. As mentioned before, it has recently been suggested that ovarian carcinomas with *p53* mutations may be less susceptible to chemotherapy-induced apoptosis, such as that induced by platinum-based drugs [81]. Several studies have indicated that mutations of the *p53* gene have a role in the development of cisplatin resistance in ovarian cancer as a result of loss of the ability of *p53* to transactivate *bax*. If this hypothesis is correct, analysis of *p53* mutations in ovarian cancers may be of interest in distinguishing patients who would benefit from chemotherapy agents that do not induce apoptosis (paclitaxel) [69, 81, 94]. A recent study used apoptosis as a measure of chemosensitivity to cisplatin and paclitaxel in four ovarian cancer cell lines; the four cell lines showed various sensitivities to these drugs, and the extent of apoptosis correlated with the sensitivity of these cell lines to both agents [25]. However, the role of *p53* in regulating the extent of tumour cell apoptosis in response to chemotherapy remains unclear, since different *p53* mutations may have different biological effects.

Telomerase activity

Telomeres are specialized DNA-protein structures found at the end of eukaryotic chromosomes. They are characterized by an array of tandemly repeated G-rich DNA sequences. As DNA polymerase is unable to replicate the very ends of linear DNA, every replication cycle leads to progressive shortening of telomeric ends in normal somatic cells. Shortening of telomeres results in chromosomal instability and cell death. Telomerase is a ribonucleoprotein enzyme that catalyses the addition of TTAGGG repeats to the telomeres. Telomerase activity has been detected in germ cells, permanent cell lines and tumours [37]. There is evidence in several types of tumour that the presence of high levels of telomerase correlates with a poor prognosis [29, 30]. Telomerase activity, measured by the TRAP (telomerase repeat amplification protocol), has been detected in 92% of ovarian carcinomas, 17% of ovarian borderline tumours and 0% of ovarian cystadenomas. Interestingly, high telomerase activity was detected in poorly differentiated carcinomas [55].

Conclusions

Our knowledge of the molecular mechanisms involved in ovarian tumorigenesis is increasing, and yet, we are not certain about the value of c-*erb* B-2 and *p53* as potential discriminators for tumours with a more aggressive behaviour. However, we do think that the study of the molecular events involved in the early stages of neoplastic transformation, particularly when interpreted within the appropriate morphological context, may provide the basis for improving the management of patients with ovarian cancer in the future.

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