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The molecular pathology of ovarian serous borderline tumors†

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Molecular studies in ovarian serous borderline tumors (OSBTs) have been used to understand different aspects of this neoplasm. (i) Pathogenesis, Kras and Braf mutations represent very early events in the tumorigenesis of OSBT as both are detected in serous cystadenomas associated with OSBTs. In contrast, serous cystadenomas without OSBTs do not show Kras or Braf mutations. In OSBTs, Kras mutations range from 17% to 39.5%, while Braf mutations range from 23% to 48%. The former is comparable with the range of Kras mutations in ovarian low-grade serous carcinomas (OLGSCa), 19%–54.5%. In contrast, Braf mutations in OLGSCa range from 0% to 33%. Serous cystadenomas appear to progress to OSBT due to a Braf mutation, but this mutation is rarely involved in the progression to OLGSCa. OSBTs with Braf mutation are associated with cellular senescence and up-regulation of tumor suppressor genes. In contrast, OSBTs without a Braf mutation may progress to OLGSCa due to Kras mutation or some other genetic alterations. (ii) The relationship between OSBTs and the extraovarian disease, a monoclonal versus mutifocal origin? This is still matter of debate as studies using different techniques have failed to settle this controversy. (iii) Biological behavior, Braf mutations appear to have a protective role against the progression of OSBT to OLGSCa, while Kras mutations are commonly seen in cases of OSBT that recurred as LGSCa. Nevertheless, LGSCa as a recurrence of an OSBT can originate from OSBTs with or without detectable Kras mutations. Also, it appears to be an association between Kras G12v mutation and a more aggressive phenotype of OSBT that recurred as LGSCa. (iv) Actionable targets, currently there are limited data. It has been reported that cancer cell lines with Kras G12v mutation are more sensitive to selumetinib than cell lines with wild-type Kras.

Key words: Braf, Kras, ovary, serous borderline tumor, serous tumor of low malignant potential, implants

introduction

Ovarian serous borderline tumor (OSBT) represents the most common type of borderline tumor arising in the ovary [1]. This neoplasm is usually associated with a serous cystadenoma or adenofibroma [2], confined to the ovary and has an indolent course [3]; however, up to 6.8% of the cases can progress to low-grade serous carcinoma (LGSCa) [4]. Certain clinicopathologic features of OSBTs, such as the presence of a micropapillary/cribriform pattern [5–7], microinvasion [8] and advanced stage at presentation[3, 9], have been linked to a more aggressive disease; nevertheless, cases without these features can be associated with recurrences or LGSCa [10–12]. Another interesting aspect of these neoplasms is represented by the unique nomenclature used to designate the extraovarian disease that can be seen in up to 30% of the cases [3]. This extraovarian disease is commonly seen as small deposits of tumor in the omentum or peritoneum (implants) or lymph nodes (involvement) [3]. The unique nomenclature used in these cases diverts from the conventional term (i.e. metastasis) used in surgical pathology to designate the secondary sites of tumor involvement when dealing with a given neoplasm. This divergent nomenclature stems from the thought that the extraovarian disease might represent an independent focus of disease which is not necessarily related to a poor outcome [13, 14]. Regarding prognosis, as indicated above, certain clinicopathologic features have been linked to a more aggressive behavior; even though, some cases lacking these features are associated with recurrences or progression to LGSCa [4, 12]. The therapeutic options for patients with OSBTs are limited. Treatment is usually given to those cases associated with invasive implants or LGSCa. Essentially, most cases have been treated with conventional platinum-based therapy and/or hormonal manipulation with limited success [15–17]. Molecular studies have been used to understand the pathogenesis, the relationship of the ovarian tumor and the extraovarian disease, prognosis and treatment in cases of OSBT. Although over a decade has passed from the initial publications covering these topics, a lot of work is still needed to unlock the many unsettled issues related to this disease.

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pathogenesis

OSBTs usually arise in the background of serous cystadenoma [2]. This histological finding strongly suggests that the former arises from the latter. In order to confirm this hypothesis, a group of investigators at Johns Hopkins studied a small cohort of eight cases of OSBT associated with serous cystadenoma. This group found that three of the SBT cases showed Braf mutations, while four SBT cases had Kras mutations. All cases with mutant Braf had wild-type Kras and vice versa. Of note, the serous cystadenoma that represented the background of these neoplasms harbor the same Kras and Braf mutations (i.e. this was the case in six of seven informative cases) [2]. A previous publication from the same institution had already found that serous cystadenomas without serous borderline tumors show no Kras and Braf mutations [18]. These findings indicate that OSBTs indeed arise from ovarian serous cystadenomas. However, additional studies are needed to determine whether mutations of Kras and Braf are enough to initiate the development of OSBTs or additional genetic 'hits' are needed in the tumorigenesis of this neoplasm [2]. OSBTs may have Braf or Kras mutations (Tables 1 and 2). Braf mutations in this type of tumor have been found to range from 23% to 48%, while Kras mutations have been reported to range from 17% to 39.5% [19–23, 26]. Also, *ER*BB2 mutations have been found in 6% of OSBTs [21]. Mutations of each of these three genes are mutually exclusive. It is thought that mutations of Kras, Braf or *ER*BB2, upstream regulators of the mitogen-activated protein kinase (MAPK), activate the MAPK signal transduction pathway and this will produce an uncontrolled proliferation. Of interest, the incidence of Kras mutations in OSBT is comparable with the incidence of Kras mutations in OLGSCa (Table 2), which ranges from 19% to 54.5% [19–23, 25, 28, 29]. In contrast, the incidence of Braf mutations in OLGSCa has been found to range from 0% to 33% [19, 22–27] (Table 1). Therefore, it seems that benign ovarian serous tumors can progress to OSBT due to a Braf mutation, but this mutation is rarely involved in the progression to OLGSCa [22]. OSBTs with Braf mutation are associated with cellular senescence and up-regulation of tumor suppressor genes [30]. In contrast, OSBTs without a Braf mutation may progress to OLGSCa due to Kras mutation or some other genetic alteration [23, 31, 32].

Table 1. Frequency of Braf mutations in ovarian serous borderline tumor (OSBT) and ovarian low grade serous carcinoma (OLGSCa)

OSBT	OLGSCa	Reference
28%	33%	Singer et al. [19]
31%	N/A	Mayr et al. [20]
48%	N/A	Anglesio et al. [21]
41%	N/A	Verbruggen et al. 2009 [43]
30%	2%	Wong et al. [22]
23%	0%	Vereczkey et al. [23]
41%	0%	Schlosshauer et al. [24]
N/A	0%	Sundov et al. [25]
45%	5.3%	Grisham et al. [26]
N/A	5.9%	Farley et al. [27]

Table 2. Frequency of Kras mutations in ovarian serous borderline tumor (OSBT) and ovarian low grade serous carcinoma (OLGSCa)

OSBT	OLGSCa	Reference
36%	NA	Mok et al. [28]
35%	33%	Haas et al. [29]
33%	35%	Singer et al. [19]
22%	N/A	Mayr et al. [20]
18%	N/A	Anglesio et al. [21]
17%	19%	Wong et al. [22]
39.5%	23%	Vereczkey et al. [23]
N/A	54.5%	Sundov et al. [25]

to assess the relationship of the ovarian tumor and the extraovarian disease

Is the extraovarian disease secondary to the ovarian tumor (i.e. monoclonal origin)? Or is it independent from the ovarian tumor (i.e. multifocal in origin)? Studies evaluating X-chromosome inactivation pattern and loss of heterozygosity (LOH) have shown conflicting results. On the one hand, a small study of eight cases of OSBT-only two with peritoneal implants showed one case sharing the same LOH pattern and the same X-inactivation pattern in the implants, but lacking LOH inactivation and showing a different X allele inactivated in the OSBT. The second case of this cohort showed the same pattern of LOH and X-inactivation in the OSBT and the implant [33]. Another study, where the pattern of X-chromosome inactivation could be determined from the OSBTs and peritoneal implants of 13 patients from a cohort of 18 patients, showed that seven cases had nonrandom inactivation of the X-chromosome and in six of these cases, the peritoneal and ovarian tumors had different inactivation patterns (i.e. supporting thus a multifocal origin) [34]. Additional studies by two different groups of investigators demonstrated the presence of identical patterns of X-chromosome inactivation and LOH supporting a monoclonal origin for the extraovarian disease [35, 36]. These conflicting results are most likely related to the limitations of these techniques to evaluate clonality as aneuploidy and abnormal methylation patterns can interfere with X-chromosome inactivation patterns, while the absence of informative markers or failure to detect LOH results in an underestimate of the frequency of clonality. Mutational analysis was supposed to generate more reliable results; however, this has not been the case and the debate between monoclonal versus multifocal disease still continues. A study of Kras and Braf mutations in 15 cases of OSBT with peritoneal implants, where pyrosequencing was used, showed genetic heterogeneity (i.e. multifocal origin). In contrast, two other groups of investigators studied the same mutations using laser-captured microdissected FFPE tissue for PCR-Sanger Sequencing or pyrosequencing in a total of 60 cases of OSBT with peritoneal implants and they found identical Kras and Braf mutations in the ovarian tumor and in the extraovarian disease (i.e. supporting thus a monoclonal origin) [37, 38].

prognosis

Braf mutations appear to be more common in OSBTs and in early-stage OLGSCa, but rare in advanced OLGSCa [22, 26, 27]. Braf mutations are more frequently detected in OSBTs that did

not recur, this finding could indicate a protective role of this mutation against progression to LGSCa [22, 26, 30]. Kras mutations are commonly seen in OSBTs that recurred as LGSCa [39]. Of interest, Kras-mutated cells can be present in very small number in the primary OSBT and their identification depends on the use of elaborate techniques such as full COLD (coamplification at lower denaturation temperature)-PCR and deep sequencing rather than the use of a more common methods such as conventional PCR and Sanger sequencing. Furthermore, LSCa after the diagnosis of OSBT can originate from tumors with or without detectable Kras mutations [39]. A study by our group showed that cases of OSBT with Kras G12v mutation had a shorter survival time from the time of the initial ovarian tumor diagnosis than cases without this mutation. This could indicate that OSBTs with Kras G12v mutation could represent a more aggressive phenotype of OSBT that recurred as LGSCa [39]. This finding contrasts with the results of a small cohort of *de novo*, advanced stage LGSCa studied by our group which showed that cases without Braf/Kras mutations had a shorter overall survival than cases with Braf/Kras mutations (47.3 versus 77.9 months; $P = 0.28$) [22] and further confirmed with a large cohort (66.8 versus 106.7 months; $P = 0.018$) [40].

treatment

Although surgery remains the cornerstone in the treatment of OSBTs, attempts to identify molecular alterations with a potential impact on treatment have been made. The available data about the impact of molecular studies on the treatment of OSBTs are limited. However, some information is available regarding the role of Kras mutations on the treatment of OSBT as it has been found that cancer cell lines with Kras G12v mutations are more sensitive to AZD6244 (selumetinib) than cell lines with wild-type Kras. Also, a small study from our group showed that two patients with LGSCa that had developed as a recurrence of OSBT and that contained Kras G12v mutations were responders to selumetinib [39]. Another LGSCa patient with complete response lasting more than 5 years had a 15-nucleotide deletion in the negative regulatory helix of the MAP2K1 gene encoding for MEK1 [41]. Although Braf mutation is rare in LGSCa, one LGSCa patient with Braf mutation did respond to a Braf inhibitor (vemurafenib) [42].

summary

Although molecular studies have contributed to our understanding of the pathogenesis of OSBTs, a lot of work is still needed to clarify the relationships of the ovarian neoplasm with the extraovarian disease, to identify prognostic indicators and to provide targeted therapy. Kras and Braf mutations appear to represent very early events in the tumorigenesis of OSBT. However, Braf mutations appear to have a protective role against the progression to LGSCa. Kras mutations are commonly seen in OSBTs that recurred as LGSCa. The identification of Kras-mutated cells depends on the use of elaborate techniques such as full COLD-PCR and deep sequencing. OSBTs with Kras G12v mutation appear to represent a more aggressive phenotype. Of interest, LGSCa developing after the diagnosis of OSBT and with Kras G12v mutation may respond to selumenitib. As we continue to use molecular studies to clarify the features of this disease that still remain unclear, attention to: (i) tumor heterogeneity, (ii) the true concordance of any given mutation (for example, Braf or Kras mutation) in the primary tumor and recurrent or metastatic disease, and (iii) the advantages and limitations of molecular techniques used, are of utmost importance to draw meaningful conclusions.

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disclosure

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