Mutations of BRAF and KRAS Precede the Development of Ovarian Serous Borderline Tumors

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ABSTRACT

Molecular genetic changes that are associated with the initiating stage of tumor development are important in tumorigenesis. Ovarian serous borderline tumors (SBTs), putative precursors of low-grade serous carcinomas, are among the few human neoplasms with a high frequency of activating mutations in BRAF and KRAS genes. However, it remains unclear as to how these mutations contribute to tumor progression. To address this issue, we compared the mutational status of BRAF and KRAS in both SBTs and the adjacent epithelium from cystadenomas, the presumed precursor of SBTs. We found that three of eight SBTs contained mutant BRAF, and four SBTs contained mutant KRAS. All specimens with mutant BRAF harbored wild-type KRAS and vice versa. Thus, seven (88%) of eight SBTs contained either BRAF or KRAS mutations. The same mutations detected in SBTs were also identified in the cystadenoma epithelium adjacent to the SBTs in six (86%) of seven informative cases. As compared to SBTs, the cystadenoma epithelium, like ovarian surface epithelium, lacks cytological atypia. Our findings provide cogent evidence that mutations of BRAF and KRAS occur in the epithelium of cystadenomas adjacent to SBTs and strongly suggest that they are very early events in tumorigenesis, preceding the development of SBT.

INTRODUCTION

It has been shown that tumors result from an accumulation of genetic alterations that result in uncontrolled cellular proliferation. Identification of the alterations that occur early in tumor development is critical to understanding carcinogenesis and can provide insight into potential markers for early detection (1–3). Ovarian cancer is one of the most lethal neoplasms in women, and serous carcinoma is the most common type (4), but the molecular events that underlie the development of ovarian serous carcinoma are largely unknown. Recent studies have shown that ovarian serous carcinoma develops along two distinct pathways, and we have proposed a model of ovarian carcinogenesis that reflects this concept (5–7). In one pathway, invasive low-grade serous carcinoma develops from a noninvasive (or in situ) tumor that has traditionally been termed “serous borderline tumor” (SBT; ref. 8). The progression of SBT to invasive low-grade carcinoma mimics the adenomacarcinoma sequence in colorectal carcinoma (1). Detailed analysis of SBTs shows that SBTs consist of two tumors at different stages of tumor progression, a benign tumor termed “atypical proliferative serous tumor,” and an intraepithelial low-grade (noninvasive micropapillary serous) carcinoma, the immediate precursor of invasive low-grade serous carcinoma (5, 7). SBTs are frequently associated with serous cystadenomas that develop from ovarian surface epithelium through a hyperplastic process (9). Like ovarian surface epithelium, the epithelial cells of a cystadenoma do not show cytological atypia, and their proliferation index is extremely low (9). In the second pathway, high-grade serous carcinoma develops from ovarian surface epithelium or from surface inclusion cysts (10), but precursor lesions have not been well characterized. Accordingly, this process has been described as “de novo” (5).

Molecular genetic analysis has shown that SBTs and invasive low-grade serous carcinomas are characterized by mutations of BRAF and KRAS in 61 to 68% of cases (6, 7, 11, 12), but p53 mutations are rare (12–14). In contrast, high-grade serous carcinomas frequently contain p53 mutations (>50%) but rarely BRAF and KRAS mutations (6, 7, 13–17). These studies analyzed advanced stage tumors in which putative precursor lesions may have been obliterated by the tumor. In this study, we confined our analysis to small SBTs and associated cystadenomas to delineate the early molecular genetic events in their pathogenesis. Specifically, we compared the mutational status of BRAF and KRAS in SBTs and the adjacent nontransformed epithelium of serous cystadenomas.

MATERIALS AND METHODS

A total of eight small SBTs (corresponding to what has been classified as atypical proliferative tumor) and the associated cystadenomas were collected. The acquisition of tumor samples was approved by the Johns Hopkins institutional review board. The SBTs ranged from 8 to 20 mm (average 16 mm) in greatest dimension and associated cystadenomas ranged from 5 to 8 cm (average 6.8 cm). The SBTs occupied 5 to 15% of the total surface area of the cystadenomas. Only a small number of cases were studied because although cystadenomas and SBTs are not uncommon, cystadenomas containing synchronous small SBTs are relatively rare. Microscopically, the SBTs contained a hierarchical branching papillae lined by epithelial cells with mild to moderate cellular atypia (Fig. 1). The epithelium of the SBTs merged abruptly with the cystadenoma epithelium that was composed of a single layer of flat to columnar cells without atypia (Fig. 1). The Palm laser capture microdissection microscope (Zeiss) was used to separately collect the epithelium from the SBTs and adjacent cystadenoma. The PicoPure DNA extraction kit (Arcturus, Mountain View, CA) was used to prepare genomic DNA. PCR was then done, and an ABI 3100 sequencer (ABI, Foster City, CA) was used to do nucleotide sequencing. Exon 1 of KRAS and exon 15 of BRAF were both sequenced as each exon harbors almost all of the mutations in both genes (6, 7, 11, 18). The primers for PCR and sequencing were as follows: for BRAF, 5′-ttggctgctcgt-agaaatgtaatgc-3′ (forward); 5′-ccacaaatggatacagacac-3′ (reverse); and 5′-gaa-agaatgtagctgttccatcta-3′ (sequencing); for KRAS, 5′-taaggcgctctgaaagctg-3′ (forward); 5′-ttggctcaggatcatatatc-3′ (reverse); and 5′-ctgccagcata-tatccataaaaaa-3′ (sequencing). The Lasergene program (DNASTAR, Madison, WI) was used to analyze the sequences.

RESULTS

The results of the mutational status correlated with the SBT or cystadenoma component of the tumors are shown in Table 1. We found that four SBTs (cases 1, 4, 5, and 8) contained activating KRAS mutations at codon 12 (three mutations of GGT to GAT and one mutation of GGT to GTT) and three SBTs (cases 3, 5, and 6) had BRAF mutations at codon 599 (all of T1796A mutation). As in our previous report (6), the presence of KRAS and BRAF mutations was mutually exclusive. Thus, seven (88%) of eight SBTs had either a BRAF or a KRAS mutation. Case 2 contained wild-type KRAS and BRAF. Analysis of the mutational status in the epithelium from the cystadenomas adjacent to the SBTs revealed that both the cystade-
noma and SBT components contained identical mutations in six of seven informative cases. Representative sequence analyses are shown in Fig. 2. The frequent mutations of \textit{KRAS} and \textit{BRAF} in small SBTs are consistent with previous reports showing mutations in either \textit{BRAF} or \textit{KRAS} in 66 to 68\% of large SBTs (6, 11). The higher frequency of mutations (88\%) in the current report is probably because of the use of purer tumor cell samples obtained by laser capture microdissection or may have resulted from the small sample size in the present analysis.

\section*{DISCUSSION}

The findings in this study provide important insights into the molecular pathogenesis of low-grade ovarian serous tumors (Fig. 3). Because we only analyzed a single time point in the sequence of cystadenoma to SBTs, we can only infer that the findings truly describe the events in early tumor progression. However, the coexistence of a cystadenoma with a SBT strongly suggests that the latter arises from the former (Fig. 3). Accordingly, the presence of identical mutations in the cystadenoma epithelium that displayed no evidence of cytological atypia strongly suggests that mutations of \textit{BRAF} and \textit{KRAS} occur before the development of a SBT and indicates that cystadenomas are the precursors of SBTs. Our results support the view that mutations of \textit{BRAF} and \textit{KRAS} (or \textit{NRAS}) are early events associated with tumor initiation as occurs in melanoma (19) and colorectal carcinoma (20).

We have recently studied 30 consecutive pure cystadenomas without SBTs and have shown an absence of \textit{BRAF} and \textit{KRAS} mutations in all of them (9). The frequency of mutations in \textit{BRAF} and \textit{KRAS} in cystadenomas associated with SBTs was significantly higher than those without SBTs ($P < 0.001$, Fisher’s exact test; Table 2). This finding together with the fact that SBTs are relatively uncommon as compared to cystadenomas (21–25) suggests that only a small proportion of serous cystadenomas are neoplastic with the potential to progress to SBTs. Finally, our findings suggest a “gatekeeper” role of \textit{BRAF} and \textit{KRAS} genes in the development of low-grade serous carcinomas (26). This is supported by the observation that activating mutations in these genes are oncogenic in experimental cell culture systems (19, 27, 28) probably through a constitutive activation of mitogen-activated protein kinase (29, 30). Future experiments will determine whether mutations of \textit{BRAF} and \textit{KRAS} are sufficient to initiate the development of SBTs or additional genetic “hits” are required in tumorigenesis. Because mutations of \textit{BRAF} and \textit{KRAS} in serous cystadenomas are associated with the development of SBTs, detection of \textit{BRAF} and \textit{KRAS} mutations could facilitate the differen-

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|c|}
\hline
\textbf{Gene} & \textbf{Case 1} & \textbf{Case 2} & \textbf{Case 3} & \textbf{Case 4} & \textbf{Case 5} & \textbf{Case 6} & \textbf{Case 7} & \textbf{Case 8} \\
\hline
\textit{KRAS} & G35A* & WT & WT & G35T & G35A & WT & WT & G35A \\
SBT & G12D† & & & G12V & G12D & & & \\
\hline
Cyst & G35A & WT & WT & & G35A & WT & WT & G35A \\
G12D & & & & & G12D & & & \\
\hline
\textit{BRAF} & WT & WT & T1796A & V599E & V599E & T1796A & T1796A & WT \\
SBT & WT & WT & T1796A & V599E & T1796A & T1796A & WT & WT \\
\hline
Cyst & WT & WT & & V599E & & & & \\
\hline
\end{tabular}
\caption{Mutational status of \textit{KRAS} and \textit{BRAF} genes in eight small serous borderline tumors and associated cystadenomas}
\end{table}

\begin{itemize}
\item Abbreviation: WT, wild-type.
\item * Alteration in nucleotide sequence.
\item † Alteration in amino acid sequence.
\end{itemize}
tiation of cystadenomas with a high risk of progression from the vast majority of cystadenomas that lack \( \text{BRAF} \) or \( \text{KRAS} \) mutations and have a very low risk of progression. Development of molecular assays (31, 32) that can detect such mutations (in cyst fluid, for example) could play an important role in the management of patients with ovarian cystadenomas, particularly young women who would prefer fertility-sparing treatment.

### ACKNOWLEDGMENTS

We gratefully acknowledge the technical support of the Oncology Imaging Core at the Johns Hopkins Medical Institutions for the photomicrographs and the laser capture microdissection.

### REFERENCES


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