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Original contribution

Spindle assembly checkpoint protein expression correlates with cellular proliferation and shorter time to recurrence in ovarian cancer☆,☆☆

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Summary Ovarian carcinoma (OC) is the most lethal of the gynecological malignancies, often presenting at an advanced stage. Treatment is hampered by high levels of drug resistance. The taxanes are microtubule stabilizing agents, used as first-line agents in the treatment of OC that exert their apoptotic effects through the spindle assembly checkpoint. BUB1-related protein kinase (BUBR1) and mitotic arrest deficient 2 (MAD2), essential spindle assembly checkpoint components, play a key role in response to taxanes. BUBR1, MAD2, and Ki-67 were assessed on an OC tissue microarray platform representing 72 OC tumors of varying histologic subtypes. Sixty-one of these patients received paclitaxel and platinum agents combined; 11 received platinum alone. Overall survival was available for all 72 patients, whereas recurrence-free survival (RFS) was available for 66 patients. Increased BUBR1 expression was seen in serous carcinomas, compared with other histologies ($P = .03$). Increased BUBR1 was significantly associated with tumors of advanced stage ($P = .05$). Increased MAD2 and BUBR1 expression also correlated with increased cellular proliferation ($P < .0002$ and $P = .02$, respectively).

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Reduced MAD2 nuclear intensity was associated with a shorter RFS (P = .03), in ovarian tumors of differing histologic subtype (n = 66). In this subgroup, for those women who received paclitaxel and platinum agents combined (n = 57), reduced MAD2 intensity also identified women with a shorter RFS (P < .007). For the entire cohort of patients, irrespective of histologic subtype or treatment, MAD2 nuclear intensity retained independent significance in a multivariate model, with tumors showing reduced nuclear MAD2 intensity identifying patients with a poorer RFS (P = .05).

1. Introduction

Ovarian carcinoma (OC) is the most lethal of the gynecological malignancies, often presenting at an advanced stage, with intraperitoneal dissemination and distant metastases. Treatment of advanced OC is hampered by eventual tumor resistance to chemotherapy. Up to 30% of patients do not respond to conventional chemotherapy, and a high proportion of initial responders (22%-59%) develop acquired drug resistance [1]. Most deaths result from metastases, which are refractory to conventional chemotherapy. Paclitaxel (Taxol) is a first-line chemotherapeutic agent effective in the treatment of OC and is used in combination with platinum agents, following optimal surgical debulking [2]. Paclitaxel is a microtubule stabilizing agent that functions primarily by interfering with spindle microtubule dynamics causing cell cycle arrest and apoptosis [3]. The mechanisms underlying its action have not, however, been fully elucidated [4]. The spindle assembly checkpoint (SAC) is a regulatory mechanism present in all eukaryotes, which prevents chromosome mis-segregation during mitosis, thereby preventing aneuploidy [5]. From a chemotherapeutic perspective, it is the checkpoint through which microtubule inhibitory drugs, such as paclitaxel, exert their effects, and reduced expression of SAC proteins such as BUB1-related protein kinase (BUBR1) and mitotic arrest deficient 2 (MAD2) are associated with acquired paclitaxel resistance in ovarian carcinoma cell lines [6,7].

The components of the SAC were first described in budding yeast and include mitotic arrest deficient proteins 1 to 3 (MAD1, MAD2, and MAD3, the latter now known as BUBR1 in humans) and "budding uninhibited by benzimidazole proteins," BUB1-3 [8-11]. A functional SAC is activated following treatment with antimicrotubule agents, such as paclitaxel due to interference with spindle assembly [12]. In this situation, a functioning SAC can impede cell cycle progression and promote apoptosis. In vitro studies have shown that alterations in certain SAC proteins compromise this function and thereby confer resistance to drugs such as paclitaxel. Specifically, BUBR1 is required for a sustained mitotic arrest in human cancer cells treated with microtubule targeting agents [13]. Importantly, small interfering RNA (siRNA)–mediated suppression of MAD2 and BUBR1 expression in paclitaxel-treated cancer cells abolishes checkpoint function and results in paclitaxel resistance in vitro [14].

BUBR1 exists in 2 forms within the cell—kinetochore-bound insoluble BUBR1, reported to be required for checkpoint function, and a putatively nonessential soluble form [15]. Previous immunohistochemical (IHC) analyses have reported cytoplasmic BUBR1 expression in a variety of normal tissues such as skin, colon, and testis [16] and nuclear expression in malignancies such as squamous carcinoma and adenocarcinoma of the pancreas [16]. Cytoplasmic BUBR1 has also been reported in urothelial cancers [17]. Positive cytoplasmic BUBR1 expression has been associated with reduced recurrence-free survival (RFS), advanced stage, high grade, and serous histology in OC [18]. Furthermore, our group has recently identified the independent association of decreased MAD2 intensity with reduced progression free survival in ovarian carcinomas of the papillary serous histologic subtype [19].

IHC localization of MAD2 has demonstrated nuclear expression in some squamous cancers and in adenocarcinoma of the pancreas and colon cancer [16]. A study of hepatocellular carcinoma demonstrated nuclear and cytoplasmic expression of MAD2 [20]. From other data, MAD2 localizes to the cytoplasm in normal gastric epithelium but to the nucleus in gastric carcinoma [21]. This is in contrast to testicular tissue, where MAD2 is overexpressed in the cytoplasm in seminoma but is more commonly localized to the nucleus in normal testicular parenchyma [22].

In relation to cellular proliferation, overexpression of BUBR1 in bladder cancer has been associated with high Ki-67 expression [17]. In addition, overexpression of spindle checkpoint proteins, including MAD2, has been associated with increased Ki-67 expression in colorectal mucosa [16].

In this study, we performed an IHC analysis for BUBR1, MAD2, and Ki-67 on a tissue microarray (TMA) constructed from a cohort of 72 OC patient tumor samples prospectively collected and displaying a variety of histologic subtypes including papillary serous, endometrioid, and clear cell carcinomas. One case had insufficient material for the quantification of MAD2. Overall, our objectives were as follows: (*a*) specifically characterize and quantify the immunolocalization patterns of key SAC proteins MAD2, BUBR1, and the proliferation marker Ki-67, in different subtypes of OC using automated (Aperio Technologies, Vista, CA) and manual assessment; (*b*) use the weighted κ measure of agreement to compare manual and automated scores to validate the automated scoring outputs; (*c*) investigate the relationship between the automated scoring