Emerging links of CD151 and integrins to tumorigenesis and EMT induction in ovarian cancer

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Ovarian cancer, primarily the epithelia-origin high-grade serous (HGS) type, is one of the most lethal gynecological malignancies. Clinically, over 75% of newly diagnosed HGS ovarian cancer patients carry stage III-IV diseases, and have less than 33% survival rate over a 5-year span. Current treatment of such aggressive disease remains largely dependent on two types of chemotherapeutic drugs: Taxane- and platinum-based agents. Thus, there is an imminent need for the improvement in the detection, diagnostic modalities and target-based therapies against this malignant disease. The majorities of HGS ovarian tumors exhibit mutational inactivation or loss of p53 and/or BRCA1/BRCA2 genes, thereby conferring strong genomic instabilities. As such, currently available target–based therapies, such as inhibitors targeting ErbB receptors or Raf/Ras/MAPK- or PI3K/Akt-dependent oncogenic pathways, display limited efficacy against HGS ovarian cancer. Our recent study suggests that the malignancy of this aggressive disease, at least in part, is regulated by CD151 and its associated laminin-binding (LB) integrins. Our clinical, functional and xenograft analyses consistently indicate that in contrast to prior reports on the RGD-based integrins, CD151-LB integrin complexes play a strong suppressive role in ovarian tumorigenesis and metastatic progression. In this short review/commentary, we will briefly summarize our current understanding of integrin function and signaling in ovarian cancer. We will discuss the emerging clinical significance and functional roles of CD151-LB integrin complexes in this disease. Finally, we will provide an outlook of how studies of CD151-integrin complexes may shape our understanding of ovarian cancer aggressiveness and facilitate the development of effective biomarkers and therapeutic targets.

Keywords: CD151; Integrin; EMT; Ovarian cancer; Slug; Wnt signaling

Overview of ovarian cancer genetics, types and malignancy

Epithelial ovarian cancer is one of the most fatal gynecologic malignancies in the United States. More than 75% of high-grade serous (HGS) carcinomas, the most common type of epithelial ovarian cancer, are diagnosed in the late and highly aggressive stages [1]. Up to now, cytoreduction (or tumor debulking) followed by adjuvant chemotherapy, i.e., a combination platinum-and taxane-based agents, is still the mainstay for management of advanced ovarian cancer. The effectiveness of such clinical treatment is often complicated by chemoresistance [2]. As such, patients with ovarian cancer face a grim 5-year survival rate that is below 33% [3,4]. Thus, there is an urgent need for the development of more effective target-based therapies to improve clinical outcomes of this disease.

Extensive genomic studies have unveiled high genetic heterogeneity in HGS ovarian tumors. In particular, ovarian tumors harbor extensive mutations or inactivation of loss of p53 and BRCA1/2 genes, giving rise to high genomic instability [5,6]. As a result, there are extensive chaotic rearrangements of chromosome or gene structure in HGS ovarian tumors, as reflected by massive gene amplification or deletion or loss of heterozygosity (LOH) [7]. In contrast, low-grade ovarian tumors are characterized by typical oncogenic changes or activation of ErbB receptors and/or Ras/MAPK and PI3K/Akt pathways [1]. The recent development of antibodies and small molecule-based inhibitors that target these oncogenic pathways provide great promise for curative treatment of this subgroup of ovarian cancer. By comparison, there is still lack of effective therapeutic strategies for overcoming the lethality of HGS ovarian cancer.

Based on gene expression profiling studies, HGS ovarian tumors can be further divided into at least four subtypes: mesenchymal, differentiated, immunoreactive, and proliferative [7]. There is increasing evidence that such heterogeneous nature of HGS ovarian tumors is closely associated with the epithelial-mesenchymal transition (EMT) program [8-12]. Like other carcinomas, HGS ovarian tumors undergo typical EMT process, which is featured with the loss of epithelial cell-associated cell-cell contacts and the gain of mesenchymal cell-linked cell-extracellular matrix (ECM) interactions [9]. At the molecular level, this process is accompanied by the altered expression of several key cell adhesion molecules, namely of the cadherin and integrin families [9]. These include the reduction of E-cadherin and a concomitant increase in ß5-integrin, N-cadherin, and their ECM ligands (e.g., fibronectin, collagens) [13,14]. In line with the pro-malignant nature of the EMT program, the mesenchymal subtype among HGS ovarian cancer is associated with strong tumor aggressiveness and worst clinical outcomes [14].

Integrins and ovarian cancer malignancy

As a family of heterodimeric adhesion receptors, integrins are widely recognized for their structural support of tissue architecture and signaling function in diverse biological and pathological processes [15]. Up to now, most integrin-related studies of ovarian cancer have focused on the RGD-based integrins, including ß3 and ß1, along with their ligands (e.g., vitronectin and fibronectin) [16,17]. Functionally, these molecules appear to promote ovarian tumor cell proliferation and progression [18,19]. Also, such functional links involve an enhanced tumor cell-ECM interactions and accelerated remodeling of their microenvironments [9,20].

By far, the strongest link of integrins to ovarian cancer malignancy is the extensive amplification of focal adhesion kinase (FAK), a pivotal tyrosine kinase downstream of integrin signaling. There is evidence that FAK is amplified in more than 30% of ovarian tumors [21,22] and knockdown or small molecule-based inhibition of this kinase effectively impairs the proliferation of ovarian cancer cells [22,23]. Also, some of these FAK inhibitors may act in synergy with chemotherapeutic agents to disrupt the malignancy of ovarian cancer [21,23]. Hence, the function and FAK-dependent signaling of the RGD-based integrins are crucial to ovarian tumorigenesis and progression.

Tumor-suppressing roles of CD151 and laminin-binding integrins in HGS ovarian cancer

The laminin-binding (LB) integrins, including ß31, ß61, ß71 and ß64 integrins, are principal adhesion receptors for normal or tumorigenic epithelial cells [24]. Compared to the RGD-based integrins, however, this subset of integrins has received limited attention regarding their functional and signaling roles in ovarian cancer. We recently attempted to fill this void by evaluating clinical significance and functional roles of these adhesion receptors in such disease, particularly in the context of their associated CD151.

CD151 is a member of the tetraspanin family and acts as a master regulator of LB integrin function and signaling in normal or tumorigenic epithelia cells. Notably, in well-transformed or malignant carcinoma cells with minimal or no expression of E-cadherin (e.g., MDA-MB-231 and HT1080 cells), CD151 actively promotes tumor cell adhesion, motility and invasion over laminin matrices [25-29]. CD151 also activates multiple signaling pathways or gene expression, including those mediated by PKC-, FAK-, Ras/MAPK, RTKs
ions of CD151 and LB integrins

There is increasing evidence, however, that CD151 and its associated LB integrin may also suppress tumorigenesis or metastasis in certain cancer types, particularly endometrial, prostate and skin cancer [8,38-40].

Our recent study with tissue microarray-based patient samples indicates that CD151 expression is negatively correlated with the aggressiveness of ovarian cancer [8]. In line with this clinical link, disruption of CD151 enhances ovarian tumor cell proliferation and growth or ascites production in immuno-deficient mice [8]. In particular, the tumors developed from the injected human ovarian tumor cells recapitulated the typical histomorphologic features of HGS ovarian cancer. Thus, our study for the first time provides crucial clinical and functional evidence of CD151 as a key player in ovarian cancer progression. Intriguingly, prior to our study, there were reports that CD151 and its associated α3β1 or α6β4 integrin promoted ovarian cancer cell invasiveness [41]. In fact, we did find that loss of CD151 impaired EGF-stimulated motility and invasion of ovarian cancer cells as seen in other cancer type [26,30]. However, our further mechanistic analyses shed new light on such dilemma of CD151-LB integrin complex function in ovarian cancer.

Several lines of evidence in our recent study support the notion that the tumor-suppressing function of CD151 in ovarian cancer is largely attributed to its strong role in stabilizing cell-cell contacts [8]. In particular, CD151 exhibits basolateral distribution in ovarian tumors or their normal counterparts (e.g., fallopian tube) (Fig. 1A). Such expression pattern is also detected in cultured HGS ovarian tumor cell lines (Fig. 1B) [8]. In fact, this type of CD151 distribution has been linked to the integrity of cell-cell contacts in other types of normal and malignant epithelial cells or endothelial cells [42-47]. Also, similar distribution and functional links have long been recognized for CD151-associated α3β1 integrin across multiple types of carcinomas [42,48,49]. Furthermore, there is evidence that α3β1 integrin is inversely correlated with the metastatic potential [50,51]. In addition, α6β4 integrin is implicated as a suppressor of tumorigenesis in the absence of oncogenic H-RAS [52]. Collectively, our data and other studies consistently suggest that during tumorigenesis and metastasis, the functions of CD151 and LB integrins are oncogenic context- and/or tumor stage-dependent.

Control of EMT and transcription factor Slug by CD151-α3β1 integrin complexes

One of the unexpected findings in our recent study is that CD151 ablation causes a partial reduction in E-cadherin expression on the surface of ovarian cancer cells (~ 33% by FACS analysis) (Fig. 1B) [8], in contrast to the prior analysis of skin squamous carcinoma cells [42]. Such observation also differs from the formation of strong mesenchymal phenotype

Figure 1. Expression and function of CD151-integrin complexes in human serous-type ovarian cancer. A IHC staining of CD151 in human ovarian tumors showing epithelia-like (Case 1) or mesenchymal-like (Case 2) morphologies. Scale bar: 100μm. B Immunofluorescence images of E-cadherin protein on the surface of OVCAR5 cells with or without Cd151 knockdown. Red: antibody staining; Green: GFP. C Schematic illustration of function and signaling of CD151-α3β1 integrin complexes in HGS ovarian cancer.
during the EMT process or tumor progression \cite{10,53}. However, our finding is consistent with the emerging notion that the EMT may exist in a spectrum form in ovarian tumors, according to recent analyses of a panel of cultured cell lines \cite{12}. Also, CD151 disruption leads to a marked increase in the expression or activation of Slug, a transcription factor known to promote the EMT phenotype during a variety of physiological or pathological processes \cite{54,55}. In fact, Slug is also widely regarded as a crucial driver of ovarian cancer development and metastasis \cite{13,20,56,57}. While multiple factors or pathways have been linked to the induction of Slug \cite{55,58}, our study suggests that the induction of Slug upon CD151 removal may be attributable to the disruption of cell-cell contacts or reduced expression of E-cadherin (Fig. 1B). Also, the change in Slug protein may result from an increased protein stability, rather than mRNA, according to our recent studies of mammary epithelial cells \cite{8,60}. Thus, our recent findings provide strong evidence that CD151-\(\alpha_3\)\(\beta_1\) integrin complexes repress the EMT process in ovarian cancer by affecting the expression or activation of transcription factor Slug.

It is also worth noting that the ECM organization in ovarian carcinoma cells is altered upon CD151 disruption, particularly a concomitant increase in fibronectin deposition \cite{42,44}. The fibronectin-\(\alpha_5\)\(\beta_1\) integrin engagement has been shown to be more effective in activating RhoA in carcinoma cells, compared to the laminin-LB integrin interaction \cite{60}. In light of the strong pro-malignant function and signaling of fibronectin and associated \(\alpha_5\)\(\beta_1\) integrin interaction in ovarian cancer \cite{18}, CD151-LB integrin complexes may also exert tumor-suppressing function by sequestering the signaling of the fibronectin-\(\alpha_5\)\(\beta_1\) integrin-RhoA axis.

**Future functional and signaling studies of CD151 and laminin-binding integrins in ovarian cancer**

*Potential feedback of Wnt signaling upon disruption of CD151-integrin complexes*. Our recent Slug-based observations also imply that CD151-integrin complexes may impact the aggressiveness of ovarian cancer at transcriptional level. In fact, we detect a strong functional link of CD151-\(\alpha_3\)\(\beta_1\) integrin complexes to the canonical Wnt signaling \cite{8}. To certain degree, this observation is in line with recent signaling analyses of CD9 and other tetraspanins \cite{35,61,62}. Intriguingly, CD151 ablation also leads to an upregulation of KIAA1199 and RNFI43, two newly identified negative regulators of Wnt signaling \cite{63,64}. Currently, we are investigating whether such molecular changes constitute a feedback loop of activated Wnt signaling in response to impaired cell-cell contacts or Slug expression upon the disruption of CD151 or LB integrins.

**Downstream effectors of CD151-integrin complexes as potential biomarkers or therapeutic target**. One of interesting findings in our ovarian cancer study is the strong upregulation of MUC5AC upon CD151 ablation \cite{6}. As a large secretory glycoprotein of the mucin superfamily, MUC5AC is strongly elevated in HGS ovarian tumors \cite{65,66}. It is also readily detectable in patient’s urinary or blood samples or condition medium of cultured ovarian cancer cells \cite{67,68}. By comparison, CA125/MUC16, a mucin serving as a biomarker for ovarian cancer diagnosis or detection, is more broadly expressed in ovarian tumors, ranging from the borderline to late-stage \cite{66}. Moreover, MUC5AC is implicated in promoting tumor cell adhesion and signaling \cite{69}. Based on these observations, we are currently testing if MUC5AC serves as a candidate diagnostic or prognostic biomarker for HGS ovarian cancer.

**Concluding remarks**

Unexpectedly, our recent study suggests that a subset of integrins and associated proteins, particularly LB integrins, and their associated CD151, play a strong suppressive role in highly aggressive ovarian cancer by impacting the EMT and tumorigenic processes (Fig. 1C). This is also one of very few functional analyses of these adhesion molecules in such disease. Importantly, our findings raise a strong possibility that genetic alteration or disruption of CD151-\(\alpha_3\)\(\beta_1\) integrin complexes may cause a concomitant activation of the canonical Wnt signaling and transcription factor Slug, thereby driving tumorigenesis and metastasis (Fig. 1C). With evidence accumulating in this direction, we will begin to gain more mechanistic insights into the unusual aggressiveness of human ovarian cancer, and develop more effective biomarkers or therapeutic strategies for the detection and treatment of such malignant disease.

**Conflicting interests**

The authors have declared that no competing interests exist.

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