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| 14. ABSTRACT The oncogenic ErbB receptors, EGFR and ErbB2, are tightly coupled with the malignancy of human ovarian carcinomas. The inhibitors that target these oncogenes, however, have received limited consideration in the treatment of this type of human cancer. To a large extent, this is due to the limited sensitivity or susceptibility of ovarian cancer cells to these therapeutic agents. Recently, it has been reported that laminin-binding (LB) integrins ($\alpha 6\beta 1$ and $\alpha 6\beta 4$) act in synergy with both EGFR and ErbB2 in human epithelia-origin cancer. This suggests that targeting LB integrins may potentially provide a novel avenue to enhance the efficacy of these ErbB inhibitors in treating human ovarian cancer. CD151, a newly discovered functional regulator of laminin-binding integrins, has also been implicated in the malignancy of several types of human cancer. We have investigated the possibility that disrupting CD151- $\alpha 6\beta 1$ and CD151- $\alpha 6\beta 4$ molecular complexes would enhance the response of human ovarian cancer cells to ErbB receptor-based therapeutic inhibitors. This was achieved by disrupting CD151- $\alpha 6\beta 1$ and CD151- $\alpha 6\beta 4$ complexes through the stable expression of CD151-specific RNA interference and evaluating the subsequent impact on malignant ovarian cancer cell behaviors, proliferation, and response to ErbB inhibitors. Upon the disruption of CD151-LB integrin complexes, there was a marked decrease in cell invasion through matrigel for a number of human ovarian cancer cell lines. In certain ovarian cancer cell lines, CD151 ablation also led to a significant inhibition of the synergy between ErbB receptors and integrin complexes as well as an increase in the sensitivity of ovarian cancer cells to ErbB inhibitors. Surprisingly, CD151 ablation appeared to affect the morphology of some ovarian cancer cell lines, leading to the dramatic change from an epithelial-like to a mesenchymal-like phenotype. Consistent with this finding was the increased proliferation of these ovarian cancer cells in the absence of CD151. Furthermore, our xenograft analyses revealed that ablating of CD151 led to accelerated ovarian tumor growth and progression in nude mice. Collectively our study provides strong evidence that CD151-LB integrin complexes mediate ovarian carcinoma cell growth and sensitivity to clinically used ErbB inhibitors. | | | | | |
| SUBJECT TERMS- ErbB receptor EGFR ErbB2 CD151 Laminin Integrin $\alpha 6\beta 1$ $\alpha 6\beta 4$ Herceptin Lapatinin xenograft model EMT Tetraspanin | | | | | |
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Critical Roles of CD151- α 6 β 1 and CD151- α 6 β 4 Integrin Complexes in Human Ovarian Tumor Progression

ABSTRACT

The oncogenic ErbB receptors, EGFR and ErbB2, are tightly coupled with the malignancy of human ovarian carcinomas. The inhibitors that target these oncogenes, however, have received limited consideration in the treatment of this type of human cancer. To a large extent, this is due to the limited sensitivity or susceptibility of ovarian cancer cells to these therapeutic agents. Recently, it has been reported that laminin-binding (LB) integrins (α 6 β 1 and α 6 β 4) act in synergy with both EGFR and ErbB2 in human epithelia-origin cancer. This suggests that targeting LB integrins may potentially provide a novel avenue to enhance the efficacy of these ErbB inhibitors in treating human ovarian cancer. CD151, a newly discovered functional regulator of laminin-binding integrins, has also been implicated in the malignancy of several types of human cancer. We have investigated the possibility that disrupting CD151- α 6 β 1 and CD151- α 6 β 4 molecular complexes would enhance the response of human ovarian cancer cells to ErbB receptor-based therapeutic inhibitors. This was achieved by disrupting CD151- α 6 β 1 and CD151- α 6 β 4 complexes through the stable expression of CD151-specific RNA interference and evaluating the subsequent impact on malignant ovarian cancer cell behaviors, proliferation, and response to ErbB inhibitors. Upon the disruption of CD151-LB integrin complexes, there was a marked decrease in cell invasion through matrigel for a number of human ovarian cancer cell lines. In certain ovarian cancer cell lines, CD151 ablation also led to a significant inhibition of the synergy between ErbB receptors and integrin complexes as well as an increase in the sensitivity of ovarian cancer cells to ErbB inhibitors. Surprisingly, CD151 ablation appeared to affect the morphology of some ovarian cancer cell lines, leading to the dramatic change from an epithelial-like to a mesenchymal-like phenotype. Consistent with this finding was the increased proliferation of these ovarian cancer cells in the absence of CD151. Furthermore, our xenograft analyses revealed that ablating of CD151 led to accelerated ovarian tumor growth and progression in nude mice. Collectively our study provides strong evidence that CD151-LB integrin complexes mediate ovarian carcinoma cell growth and sensitivity to clinically used ErbB inhibitors.

Subject terms

ErbB receptor EGFR ErbB2 CD151 Laminin Integrin α 6 β 1 α 6 β 4 Herceptin Lapatinin xenograft model EMT Tetraspanin

Introduction

The oncogenic ErbB receptors, EGFR and ErbB2, contribute to the malignancy and progression of several types of human cancer, including ovarian cancer^{1,2}. The inhibitors targeting these oncogenes, alone or combined with other chemo-therapies, have been used increasingly for clinical treatment of human cancers. In contrast, the application of these therapeutic agents has received limited consideration for human ovarian cancer^{1,2}. To a large extent, this is related to the limited sensitivity of human ovarian cancer cells to ErbB inhibitors, but on the other hand, a

number of cell adhesion molecules have been shown to play important roles during human ovarian tumor growth, dissemination and metastasis¹. As principle adhesion receptors for epithelial cells, laminin-binding (LB) integrins, including $\alpha6\beta1$ and $\alpha6\beta4$, are linked to human carcinoma progression and also appear to act in synergy with ErbB receptors to drive the malignancy of human carcinomas³. In spite of this, these integrins have not been studied for their sensitivity to ErbB inhibitors and roles in ovarian cancer progression.

More recently, CD151, a member of the tetraspanin family, has emerged as a key regulator of LB integrins in human cancer cells and contributor to the invasion and metastasis of several epithelia-origin human cancers^{4,5}. Since the majority of human ovarian cancers are of epithelium origin, it is conceivable that CD151-LB integrin complexes might also play important roles in human ovarian cancer progression.

We have investigated this possibility through the use of an in vitro culture system and animal xenograft models. Our study examined the impact of the disruption of CD151-LB integrin complexes on ovarian tumor cell behaviors and proliferation. In addition, we assessed the synergy of CD151-integrin complexes and oncogenic ErbB receptors as well as sensitivity to ErbB inhibitors in representative human ovarian cancer lines. Finally, we carried out ex vivo analyses to determine if the in vitro effect of ablating CD151-integrin complexes could be recapitulated by the use of xenograft animal models.

Body

Task I. To assess the relevance of CD151-LB integrin complexes to human ovarian cancer, we first analyzed the expression of CD151 and LB integrins in a panel of human ovarian cancer cell lines by performing flow cytometry analyses. The tested cell lines were HOSE (cells derived from human ovarian surface epithelium), OVCAR-3, CAROV3, SK-OV-3, and others. As shown in [Fig. 1.](#), in contrast to HOSE cells, only a subset of ovarian cancer cell lines, including CAROV3 and SK-OV-3, expressed LB integrins such as $\alpha6\beta1$ and $\alpha6\beta4$ while CD151 was ubiquitously present. Based on this information, we selected the ovarian cancer cell lines expressing LB integrins as our models to perform subsequent functional analyses. With the use of CD151-specific shRNA developed in our lab, we ablated the CD151 protein in multiple ovarian cancer lines. Through the monoclonal antibody- and GFP-based cell sorting on flow cytometry, we obtained stable knockdown ovarian cancer lines where, as verified by western blotting, CD151 proteins were ablated or removed by more than 90%. These newly constructed lines, in combination with their control shRNA-treated counterparts, were then examined for cell invasion, morphology and proliferation. In several examined human ovarian cancer cell lines, including OVCAR-8 and CAROV3, CD151 removal markedly inhibited tumor cell invasion in response to 10 ng/ml EGF stimulation. This result indicated that disrupting CD151-LB integrin complexes affected EGF/EGFR signaling and function in human ovarian cancer cells. On the other hand, when we compared the morphology of ovarian cancer cells with or without CD151 removal, we noticed a marked EMT-like morphological change. This observation suggests that CD151 alone or in combination with LB integrins, may play a role in suppressing human ovarian tumor cell proliferation. We subsequently tested this possibility by evaluating the impact of CD151 removal on the proliferation of one representative ovarian cancer cell line. As shown in [Fig. 2.](#), our data showed that in the absence of CD151, these cancer cells grew significantly faster than in the control or parental lines. Based on this finding, instead of examining MMP we performed gene

expression profile analyses for the control and CD151-knock down ovarian cancer cell lines to determine if there were alternations in expression of signature genes corresponding to the EMT-like phenotype. Our DNA array data revealed that compared to the control, expression of a number of genes associated with mesenchymal phenotype, including vimentin and fibronectin, was markedly increased in CD151-ablated cells. When compiled, our data suggested that CD151 not only modulates EGF-based tumor cell invasion, but also affects ovarian tumor progression through its impact on ovarian cancer cell proliferation and morphology.

Task II. We also investigated whether the disruption of CD151-integrin complexes affected sensitivity or susceptibility of ovarian cancer cells to the treatment of clinically available ErbB inhibitors. First, we tested the effect of Herceptin, a clinically used monoclonal antibody for blocking the function of ErbB2 receptor. Human ovarian cancer cells were plated accordingly on laminin-coated culture dishes, treated with a wide range of Herceptin dosages, and evaluated by measuring cells proliferation index using standard PICO-green assay. Over the 72 hours of drug treatment, we noted that in comparison to the control, there was a significant reduction in ovarian cancer cell proliferation in the cell lines with an absence of CD151, while other lines, such as SK-OV-3, exhibited little response.

Next we evaluated the effect of Lapatinib, a small molecule and inhibitor that targets ErbB2 and other members of ErbB family. In our experiments we found that Lapatinib treatment led to the marked inhibition of ovarian cancer cell proliferation when CD151 protein was ablated. This decreased cell proliferation also became greater as Lapatinin concentration increased from 10 nM to 10 μ M. Together, our data suggested that disrupting CD151-integrin complexes can affect ovarian cancer cell sensitivity or susceptibility to ErbB2 inhibitors (Herceptin and Lapatininb), which provides a novel molecular basis for improving the clinical efficacy of these biology-based inhibitors in the treatment of human ovarian cancer.

Task III. Here we applied an animal xenograft model to address the question of whether CD151-integrin complexes had any in vivo role in ovarian tumor growth and progression. One of the ovarian cancer lines with robust expression of CD151 and LB integrins was selected as our model. During our construction of CD151-ablated cells, the GFP protein was used as a selection marker. This restricted our use of the Xenogen machine for assessing tumor growth in mice, just as luciferase activity detection may be restricted by the green fluorescence protein. Thus, instead of measuring tumor growth with luciferase-based in vivo imaging technology, we chose to monitor tumor growth in mice with the use of standard caliber measurement. In accordance with this procedure, immune-deficient nude mice were subcutaneously injected with 1×10^6 control and CD151-ablated ovarian cancer cells. There were 10 animals per group, and the injection was followed by the regular recording of tumor appearance and mouse survival as well as the measurement of tumor size. As shown in Fig. 3, over a span of 39 days, 4-10 mice from both groups developed tumors. However, tumors from the mice injected with cells expressing little CD151 protein grew significantly faster than those of the mice in the control group. This result suggests that CD151-LB integrin complexes suppress tumor growth and progression in human ovarian cancer, which is consistent with our in vitro data described above.

Accomplishments:

Disruption of CD151-LB integrin complexes enhance the response of human epithelia-origin ovarian cancer cells to clinically used ErbB2 inhibitors.

Key reportable outcomes:

Disruption of CD151-LB integrin complexes enhances the response of human epithelia-origin ovarian cancer cells to clinically used ErbB2 inhibitors.

Conclusion:

CD151-LB integrin complexes play critical roles in human cancer by enhancing response to ErbB2-based therapeutic agents, and promoting ovarian tumor growth and proliferation.

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Appendices:

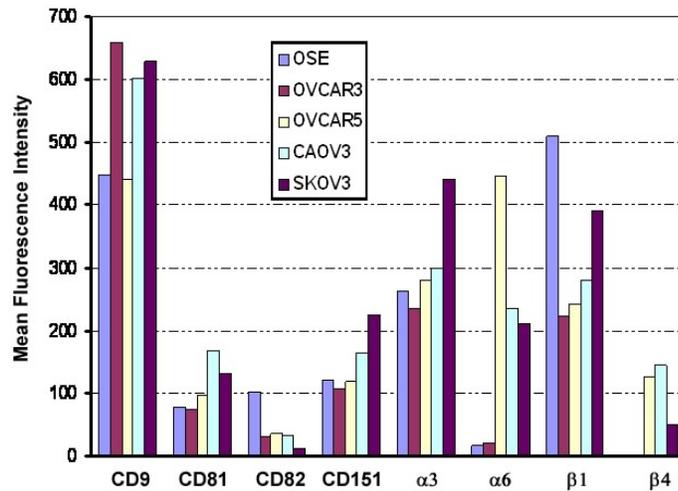


Fig. 1. Expression of CD151 and LB integrins in human ovarian cancer cell lines. Semi-confluent human ovarian cancer cells were detached through the use of non-enzymatic buffer stained with CD151 and integrin specific monoclonal antibodies followed by FITC-conjugated secondary antibodies prior to flow cytometry analyses. Surface expression was also measured for other tetraspanin molecules to serve as internal controls.

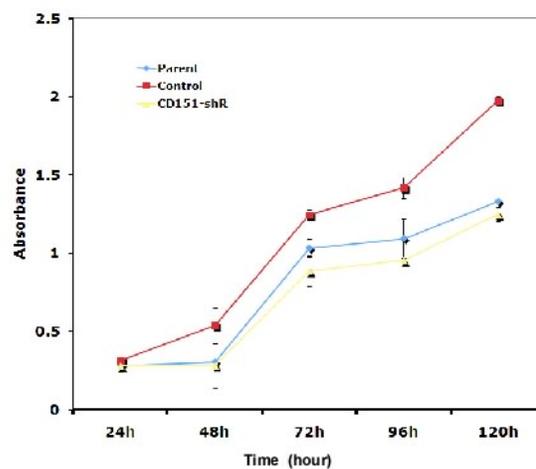


Fig.2. Impact of disrupting CD151-LB integrin on human ovarian cancer cell proliferation. Human ovarian cancer cells expressing control or CD151-specific shRNA were seeded into 24 well plates in triplicates at 5×10^5 per well. Cells were grown in DMEM containing 10% FBS, and cell numbers were determined by measuring total DNA content with MTT assay.

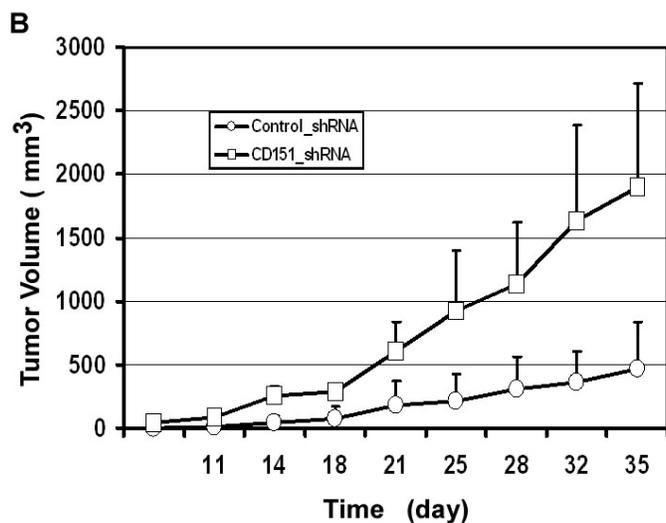


Fig. 3. Effect of CD151 ablation on human ovarian tumor growth in nude mice. Immuno-deficient nude mice were injected in back flank regions with 1×10^6 control and CD151-ablated ovarian cancer cells followed by the observation of tumor appearance and measurement of tumor size. When tumors reached 2 cm, tumor-bearing animals were removed. Tumor volumes were calculated according to the formula of length x width x height x 0.52.

Supporting Data: *none*

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