Met and the microenvironment
New insights for ovarian cancer metastasis

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Ovarian cancer often has few symptoms, which makes it difficult to detect at an early stage. Therefore, most of the women will already have metastasis at the time of diagnosis. In their search of uncovering the mechanisms underlying ovarian cancer invasion, Mitra and collaborators demonstrate that the fibronectin receptor (α5β1-integrin) can directly activate the receptor tyrosine kinase Met, independently of its ligand.

By linking the extracellular matrix with Met activation, and the invasion of ovarian cancer cells, Mitra et al. confirm the crucial role played by Met in ovarian cancer and open new perspectives in the development of ovarian cancer targeted therapies.

With 21,880 new cases estimated in 2010 and 13,850 deaths, ovarian cancer is one of the most lethal cancers for women in the United States (National Cancer Institute, www.cancer.gov/cancertopics/types/ovarian). Because most of the deaths occurred at advanced stages of the disease, it is crucial to develop a better understanding of the mechanisms leading to the spreading of ovarian tumor cells. This has been the focus of the Ernst Lengyel laboratory for several years now.

In their recent work published in Oncogene, Mitra et al. demonstrate a novel pathway linking the fibronectin receptor (α5β1-integrin) to Met. Both α5-integrin and Met have previously been reported to induce invasion in models of ovarian cancer invasion, and to be potential therapeutic targets.1,2 In the path of several reports showing that the receptor tyrosine kinase Met can be activated by other receptors tyrosine kinases (RTK), this report demonstrates that α5-integrin can directly activate Met, independently of its ligand, the hepatocyte growth factor/scatter factor (HGF/SF).

α5β1-Integrin is a Key Player for Early Metastasis In Vivo

As previously reported by the same group,3 the authors confirmed that blocking α5β1-integrin decreased the tumor burden and the number of metastasis in ovarian cancer cells. They further investigated whether this effect was due to an inhibition of the early stage of invasion, i.e., whether the fibronectin receptor played a role in the initial invasion/attachment of the cells occurring at early stages of invasion. The authors injected one million SKOV3ip1 ovarian cancer cells in the peritoneum of female athymic nude mice while simultaneously treating them with a single dose of a monoclonal antibody blocking human α5β1-integrin (Hu α5Ab; 10 mg/kg). After 35 days, the tumor weight of animals treated with Hu α5Ab was about 80% lower than the tumor weight of animals treated with control IgG. Furthermore, the number of metastasis was drastically decreased in the Hu α5Ab treated group. Interestingly, used in a similar manner, the murine antibody blocking α5β1-integrin (Mu α5Ab) did not show any effect on either tumor weight or metastasis, suggesting that the effect observed with Hu α5Ab was due to a direct effect of the antibody to the tumor cells and not to an inhibition of the vasculature.

These data, as well as supporting in vitro data, led the authors to investigate...
further the molecular mechanisms by which αβ₅-integrin induces ovarian cancer cells invasion and attachment.

**Integrin-Mediated Adhesion Activates Met, Independently of HGF/SF**

Met is a known therapeutic target for ovarian cancer due, at least in part, to its role in tumor cell invasion.⁵⁶ Because of the expertise of Lengyel’s laboratory in Met activation in ovarian cancer, and of the fact that integrins can activate RTKs, the authors logically looked at the activation of Met after transient knock-down of the fibronectin receptor by siRNA or Hu α(ab) treatment in HeyA8 cells. In both cases, Met activation was inhibited, as seen by the decreased tyrosine phosphorylation status of Met. Furthermore, immunofluorescence and coimmunoprecipitation experiments demonstrated that Met and αβ₅-integrin colocalize within the cell and form a direct interaction. However, the inhibition of Met after Hu αA treatment was rescued by addition of HGF/SF, suggesting that the fibronectin-mediated Met activation is independent of HGF/SF, and that HGF signaling is independent of fibronectin.

**Src/FAK Activation is Dependent on Fibronectin-Mediated Activation of Met**

Mitra et al. then investigated how the fibronectin-mediated Met activation affected cellular function, especially what downstream signaling pathways were activated. The focal adhesion kinase (FAK) and Src are cytoplasmic tyrosine kinases that are involved in integrin signaling.⁷⁸ Using Hu α(ab) in HeyA8 and SKOV3ip1 cells, the authors showed that both FAK and Src were phosphorylated in part through αβ₅-integrin activation. Specific knockdown of Met by siRNA in HeyA8 cells plated on fibronectin decreased both FAK and Src phosphorylation, suggesting that FAK and Src are downstream of both αβ₅-integrin and Met. The authors then looked at the effect of HGF/SF on FAK and Src phosphorylation after Hu α(ab) treatment. As expected, the inhibitory effect of the antibody on FAK and Src was reversed by HGF/SF, suggesting that Met can be activated in a ligand-dependent or -independent manner. Finally, immunoprecipitation experiments strongly suggest that Src, but not FAK, can directly interact with Met, suggesting that Met might potentially activate Src which subsequently activates FAK.

**Conclusion**

In this report, the authors discovered a common pathway for Met and fibronectin to induce metastasis through an αβ₅-integrin/Met/Src/FAK pathway, independently of the Met ligand. These results propose an explanation to how ovarian cancer cells might adapt and survive while spreading into the abdominal cavity. Therefore, this report contributes significantly to the field of ovarian cancer research.

Some questions remain open however. For instance, one can ask what the roles of PI3K/Akt and Erk pathways during early stage invasion of ovarian cancer cells are. Although those pathways have been previously identified as therapeutic targets,⁹¹⁰ in this report, activation of Met by fibronectin, unlike HGF/SF, did not activate Akt or Erk. We can then wonder whether activation of Akt/Erk pathways would be beneficial or not to the early stage attachment/invasion of those cells. Although it is possible that in physiological conditions, both HGF/SF and fibronectin activate Met (and therefore Src/FAK, Akt and Erk pathways), it is also reasonable to speculate that different stages of the invasion process might require different activation patterns of those signaling pathways. Therefore, it would be interesting to investigate further the molecular mechanisms underlying each step of invasion of ovarian cancer cells.

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