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BLADDER CANCER

Rho-sensitive pathway mediates metastasis

New data have detailed a signalling pathway involved in metastasis suppression in bladder cancer — the osteopontin–CD44–T cell lymphoma antigen (TIAM1)–Rac1 axis is a RhoGDP dissociation inhibitor 2 (RhoGDI2)-sensitive pathway that could be targeted to inhibit the development of metastasis.

RhoGDI2 acts as a metastasis suppressor in bladder cancer, and reduced expression is associated with decreased survival in patients with this disease. *In vitro*, metastatic bladder cancer cell lines UMUC3 and T24T express negligible levels of RhoGDI2 compared with the nonmetastatic T24 and RT4 cell lines, and metastatic cell invasion is stimulated by macrophage-conditioned medium.

Ahmed and colleagues engineered metastatic cell lines to express RhoGDI2 to the same levels as nonmetastatic cell lines and these cells exhibited reduced cell invasion when treated with macrophage-conditioned medium. Similarly, knockdown of RhoGDI2 in nonmetastatic cells conferred invasive behaviour when treated with conditioned medium.

The investigators identified the macrophage-secreted molecule osteopontin as the major active component in macrophage-conditioned medium affecting cell behaviour. When osteopontin was depleted from condition medium, cell invasion by UMUC3 cells decreased by 68% and recombinant osteopontin alone was sufficient to induce an invasive response. In nonmetastatic cells, knockdown of RhoGDI2 induced invasive behaviour on stimulation by osteopontin, indicating that RhoGI2 supresses osteopontin-induced cell invasion.

The CD44 isoform CD44s was identified as the receptor that mediates conditioned-medium and osteopontin-induced invasion, but CD44 expression was not affected by RhoGDI2 expression. CD44s interacts with TIAM1, and when TIAM1 was knocked down in UMUC3 cells, the invasive response to conditioned medium and osteopontin was reduced. Cells expressing mutated CD44s exhibited reduced invasive behaviour, which was rescued when cells were reconstituted with wild-type CD44s.

Rac1 activity was observed in macrophage-conditioned osteopontin-containing medium, which was inhibited by knockdown of CD44. RhoGDI2 expression blocked the effects of CD44s and macrophage-conditioned and osteopontin-containing medium on Rac1 activity, suggesting that this pathway is a RhoGDI2-sensitive axis that activates Rac1, promoting bladder cancer cell invasion.

When plated at single-cell density, UMUC3 cells stimulated with macrophage-conditioned medium formed colonies, which was inhibited by osteopontin depletion, TIAM1 knockdown, and functional blocking of CD44. Clonal growth was also suppressed by RhoGDI2 expression, whereas knockdown of RhoGDI2 in nonmetastatic cells induced clonal growth on treatment with conditioned and osteopontin-containing medium.

In vivo, stable knockdown of CD44 reduced the development of lung metastasis, as did cotreatment with a CD44 function-blocking antibody. Delayed antibody treatment also resulted in a reduction in metastasis formation.

In patients, osteopontin expression was observed to be increased in non-muscle-invasive tumours and high expression was associated with poor patient outcomes. Expression was higher in metastatic tumours than nonmetastatic primary tumours from the same patient, as was macrophage infiltration. These data suggest that this pathway is involved in clonal growth in bladder cancer.

The discovery of this pathway identifies promising targets for inhibiting progression to metastasis in patients with non-muscle-invasive bladder cancer.

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