Commentary

Rac1 and EMT: a dangerous liaison?

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Rac1 was originally identified in 1989 in human platelets as a substrate of the botulinum toxin from which its name derives: Ras-related C3 botulinum toxin substrate (1). Rac1 belongs to the Rho family of small guanosine triphosphatases (GTPases), and is ubiquitously expressed. It exists in two conformational states, an inactive GDP-bound form and an active GTP-bound form. The transition from one form to another occurs upon stimuli and depends on GTPase-activating proteins (GAPs), which inactivate Rac1, and guanine nucleotide exchange factors (GEFs), which convert Rac1 to its active form. Among GEFs, the trimeric complex SOS1/EPS8/ABI1 plays a role in Rac regulation. Dissection of the complex shows that SOS1 is central because it supports the catalytic component of the complex, and both ABI1, also named EPS8 SH3 domain binding protein, and EPS8 with its SOS1 binding domain, allow the cohesion of this complex. Functionally, Rac1 was the subject of numerous studies highlighting diverse and broad cellular functions since its discovery. Rac1 serves as a conformational switch in several signal transduction pathways at the origin of its biological functions. One of its primary function identified, far away the most characterized, is the regulation of actin cytoskeleton organization and dynamics, in the migratory structures such as filopodia and lamellipodia. Subsequently, other Rac1 functions were evidenced including roles in cell polarity, gene expression, cell-cycle progression, and cell survival.

EMT is a reversible dynamic process during which epithelial cells gradually adopt structural and functional characteristics of mesenchymal cells. In cancer, EMT is an early event of metastasis that contributes to tumor cell migration and invasion from the primary tumor (2,3). Major EMT steps comprise modifications of gene expression allowing concurrently epithelial phenotype repression and mesenchymal phenotype activation. The first changes take place at the adherens junctions with a deregulation of two main components, E-cadherin and β-catenin. One mechanism by which E-cadherin is down-regulated occurs via EMT-inducing transcription factors (EMT-TFs), which comprise three main families: SNAIL, ZEB and TWIST. EMT-TFs primarily regulate E-cadherin expression by repressing its promoter, but they also regulate in a positive manner the expression of genes associated with mesenchymal phenotypes including N-cadherin, vimentin, fibronectin, α-smooth muscle actin (α-SMA) and matrix metalloproteinases (MMPs). A reorganization of the epithelial actin cytoskeleton also takes place with the formation of several migratory structures and the expression of MMPs to degrade the extracellular matrix (ECM). Thus, the acquisition of a mesenchymal-like phenotype endows tumor cells with invasive properties, enabling them to spread toward other territories.

Fang *et al*. investigated the role of Rac1 in the regulation of EMT in ovarian cancer cells (4). In this study, the authors identify Rac1 as a potent activator of EMT, acting through two major signaling pathways, ERK/MEK and Src. Earlier studies have suggested that Rac1 may play a role in EMT by regulating several steps of this process. It was shown that Rac1 is a potent regulator of E-cadherin-mediated cell-cell adhesion by favoring the clathrin-independent endocytosis of E-cadherin (5). Once E-cadherin localization is modified, *i.e*. delocalized and/or down-regulated, cell-cell junctions are disrupted and cells scatter, as it appears when constitutive activated Rac1 is introduced in ovarian cells. In these cells, E-cadherin is

drastically down-regulated at protein level and not anymore expressed in the cell, inevitably disturbing cell-cell junctions. β-catenin was not explored in Fang's study although it is a privileged partner of E-cadherin in cell adherens junctions. The choice of not studying β-catenin is obscure, but it is easy to anticipate that β-catenin is also down-regulated at the plasma membrane of ovarian cancer cells overexpressing activated Rac1. Rac1 has been shown to control β-catenin level and phosphorylation in colon cancer through one of the Rac1 effectors, the Ser/Thr kinase p21-activated kinase PAK1. By phosphorylating β-catenin, Rac1/PAK1 axis stabilizes the protein and increases its transcriptional activity (6). Once localized into the nucleus, β-catenin is able to upregulate the transcription of EMT-TF ZEB1 (7). In parallel to the down-regulation of the major epithelial marker E-cadherin upon Rac1 activation in ovarian cancer cells, the well-known mesenchymal marker vimentin was up-regulated, and the expression of EMT-TFs Twist and ZEB1/2 was increased, evoking with certainty the establishment of EMT (4). EMT-TFs are likely to be responsible of the down-regulation of epithelial markers such as E-cadherin and the up-regulation of mesenchymal markers including vimentin (8). More recently, a link between EMT and the acquisition of cancer stem cell properties has been emphasized in cancer (9) within a particular contribution of ZEB (10,11). To our knowledge, Rac1 has not yet been studied in this context. However, it was shown that Rac1 contributes to stem-like cell maintenance in human glioma (12) and its inhibition suppresses cancer-stem cell proliferation in non-small cell lung cancer (13). In a non-cancer context, depletion of Rac1 from adult mouse epidermis leads to rapid depletion of stem cells, suggesting indeed a potential role of Rac1 in the expansion of the stem cell compartment (14).

As expected, phenotypic and expression changes of ovarian cancer cells evidencing an EMT are functionally transduced by an increased of cell invasiveness. In contrast, Rac1 down-regulation in mesenchymal-like ovarian cell lines results in an epithelial phenotype appearance with an induction of E-cadherin and a repression of vimentin expressions, along with a decline of invasiveness capability. In this picture, the authors investigated one of the major regulators of Rac1, the trimeric complex SOS1/EPS8/ABI1 that fulfills the function of GEF allowing the activation of Rac1. Interestingly, it was previously shown in two independent studies that SOS1/EPS8/ABI1 coexpression (15) and Rac1 expression (16) correlate with advanced disease stage and shorter survival of ovarian cancer patients, indicating a link between the two pathways and a pejorative impact of SOS1/EPS8/ABI1/Rac1 axis in ovarian cancer progression. To study the function of the trimeric complex SOS1/EPS8/ABI1 in EMT of ovarian cancer cells, the authors dissected each protein of the tri-complex. Silencing of each protein individually leads to an induction of E-cadherin expression along with a reduction of vimentin level. In agreement, their forced expression resulted in the opposite effect, i.e., EMT, which is fully consistent with the data obtained with constitutive activated Rac1 supporting a positive role of Rac1 in the implementation of EMT and invasive properties in ovarian cancer cells. Cell invasiveness is not the only functional manifestation of the EMT. EMT is a process by which cancer cells escape defense mechanisms such as senescence, apoptosis and anoikis (17) thus favoring tumor progression. These data correlate with human situation where patients with epithelial-like ovarian tumors show a better overall survival (17). While Rac1 was associated with EMT features as demonstrated by Fang's study (4) and others (16), Rac1 was also involved in cell proliferation. Rac1 silencing reduced not only EMT but also the oncogenic activity and proliferation of ovarian cancer cells (16). Acquisition of mesenchymal traits during EMT process of cancer epithelial cells can compromise therapeutic response to chemo- or targeted therapies. Indeed, several studies have emphasized a link between EMT and drug resistance in cancer including ovarian cancer. It is generally accepted that cancer cells with an epithelial-like phenotype are more sensitive to drugs that those with a mesenchymal-like phenotype. In the particular case of ovarian cancer, several cell lines were tested with respect to their epithelial and mesenchymal status and unexpectedly, ovarian cancer cell lines with epithelial status displayed higher resistance to cisplatin treatment than those with mesenchymal status (18). One explanation was an enrichment of cell cycle-related gene sets and apoptosis impairment in epithelial-like cells (18).

According to Fang's study, two signaling pathways were involved in Rac1-dependent activation of EMT in ovarian cells, ERK/MEK and Src (4). Both pathways led to the activation of EMT in these cells as demonstrated by the use of pharmacological inhibitors of MEK and Src tested *in vitro* and *in vivo*, suggesting that inhibitors of Rac1 downstream pathways can block EMT. Small-molecule inhibitors targeting Rac1 have been developed and could represent a better way to brake specifically the multiple actions of Rac1 (19). They could target Rac1 not only in tumor cells by inhibiting EMT and cell migration but also in microenvironment cells including endothelial cells in which Rac1 has tumor-related angiogenesis actions.

In conclusion, Rac1 is located at the center of a signaling pathway network , which is essential for tumor progression. Fang's study reinforces this role, in particular by demonstrating Rac1 involvement in EMT, here in the ovarian cancer. This study, and others, open perspectives on anti-Rac1 targeted

treatment for metastatic cancer with high Rac1 activity.

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Footnote

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