MOLECULAR & CELLULAR ONCOLOGY
2020, VOL. 7, NO. 6, e1803029 (3 pages)
https://doi.org/10.1080/23723556.2020.1803029

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AUTHOR'S VIEWS

Unconventional role of RAC1 in MET-driven anchorage-independent tumor growth

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ABSTRACT

We reported that RAC1 is a master regulator of cell migration and anchorage-independent growth, downstream of the oncogenic Receptor Tyrosine Kinase (RTK) MET. RAC1 growth-promoting role is guanosine triphosphatase (GTPase)- and phosphatidylinositol 3-kinase (PI3K)-independent but promotes mammalian target of rapamycin (mTOR) signaling through triggering its plasma membrane localization.

ARTICLE HISTORY
Received 20 July 2020
Revised 23 July 2020
Accepted 24 July 2020

KEYWORDS
MET; RAC1; PI3K; mTOR

Receptor tyrosine kinases (RTKs) are major cancer targets, but the use of RTK inhibitors in cancer patients often results in treatment resistance and the spread of the cancer to new location (metastasis). A better understanding of how RTKs induce cancer cell growth and spread is essential to design better-targeted therapies and hopefully overcome the development of resistance.

The Receptor Tyrosine Kinase (RTK) MET (also known as hepatocyte growth factor receptor (HGFR) or c-Met) is over-expressed or mutated in multiple types of cancers. How MET, and more generally RTKs, orchestrate their numerous signaling pathways to trigger specific cellular functions such as cell growth or migration remains poorly understood. In the current model of RTK signaling, among other signals, phosphatidylinositol 3-kinase (PI3K) promotes both cell migration and growth while the small guanosine triphosphatase (GTPase) RAC1 controls cell migration.

The PI3K–AKT signaling pathway is well known to be downstream of MET.[1] The PI3K family is constituted of eight isoforms divided into three classes based on their regulatory subunit, their catalytic subunit, and their substrate. The class I PI3K, the most studied, includes four isoforms: p110-alpha and p110-beta, which are ubiquitously expressed, and p110-delta and p110-gamma, which are mainly expressed in immune cells. Lately, efforts were made to develop PI3K isoform-specific pharmaceutical inhibitors, in the hope to increase efficacy and reduce side effects. Which PI3K isoform(s) is/are involved downstream of MET had yet to be discovered.

We, therefore, set to investigate the role of PI3K in oncogenic MET dependent cell migration and anchorage-independent growth.[2] We used two different oncogenic MET models: MET with point mutations in the kinase domain, M1268T and D1246N, reported in cancer patients. We have previously discovered that, when expressed in NIH3T3 cells, these mutants are oncogenic not only because they are constitutively activated but also because they signal on endosomes;[3,4] Wild-type MET activated through an autocrine loop in U87MG glioblastoma cells.

We observed that class I PI3K isoforms p110-alpha and p110-beta are required for oncogenic MET dependent cell migration, but, unexpectedly, not for anchorage-independent growth.

The investigation of how oncogenic MET induces anchorage-independent growth independently of PI3K led to the discovery of a new signaling pathway: Oncogenic MET-RAC1-mammalian target of rapamycin (mTOR). Thus, anchorage-independent growth driven by oncogenic MET requires mTOR complex 1 (mTORC1) activity, as identified using the mTORC1 inhibitor rapamycin. The complex mTORC1 is traditionally described as being regulated by PI3K activity and being part of the signaling pathway PI3K-AKT-mTOR. Instead, we found that the knockdown of the Rho-GTPase RAC1 led to the reduction of oncogenic MET-dependent mTORC1 activation, signaling, and subsequent anchorage-independent growth. RAC1 and Rho-GTPases are mostly known for their role in cell motility; however, other studies have reported they can regulate cell growth, cell cycle, and tumorigenesis.[5–8] Moreover, consistent with our study, Saci et al.[7] previously reported the role of RAC1 in serum-induced cell growth through the control of mTOR signaling.[7]

RAC1 activation requires the exchange of guanosine diphosphate (GDP), bound to RAC1 when inactive, to guanosine triphosphate (GTP). Such exchange is promoted by guanine nucleotide exchange factors (GEFs). However, the inhibition or knockdown of Vav2 and Tiam1, two GEFs mediating oncogenic MET dependent RAC1 activation,[3,9] did not affect oncogenic MET dependent anchorage-independent growth. Moreover, the overexpression of a kinase-dead RAC1-T17N construct had no effect either, even though it inhibited oncogenic MET induced cell migration (consistent with RAC1 classical role).[3,9] We observed that oncogenic MET triggered an increased localization of RAC1 at the plasma membrane, as we have previously shown,[3] but also of mTOR. Although previously reported,[7] this is an unusual location for mTOR, mainly shown to be active in the lysosome. Furthermore, siRNA knockdown of RAC1 abrogated the localization of mTOR at the plasma membrane.

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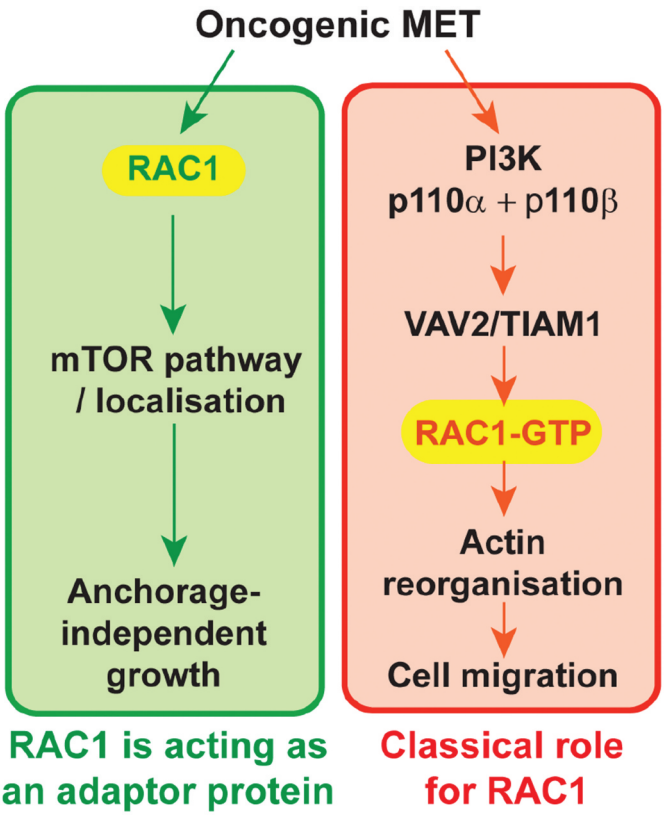

Figure 1. RAC1 is a master regulator of MET-driven migration and anchorage-independent growth. Red box: RAC1 drives actin reorganization (loss of stress fibers), leading to an increase in cell migration. Here, RAC1 has a classical role, its guanosine triphosphatase (GTPase) activity is required and is activated by class I phosphatidylinositol 3-kinase (PI3K) isoforms p110-alpha and p110-beta and the guanine nucleotide exchange factors (GEFs) VAV2/TIAM1. Green box: RAC1 acts as an adaptor protein that associates with mammalian target of rapamycin (mTOR) and promotes its translocation to the plasma membrane, leading to an increase in anchorage-independent growth.

Further investigation revealed that RAC1's binding motif RKR, within its C-terminal region, played a central role in the control of oncogenic MET dependent mTOR signaling and anchorage-independent growth. Indeed, the mutation of RKR to AAA in the RAC1-T17N construct inhibited MET dependent anchorage-independent growth and mTOR localization at the plasma membrane. Immunoprecipitation studies showed that oncogenic MET drives the association of mTOR and RAC1 in a complex. This co-recruitment is dependent on the RKR domain but independent of RAC1 GTPase activity. We concluded that RAC1 promotes oncogenic MET dependent anchorage-independent growth through playing the role of an adaptor which drives mTOR change of localization, impinging on its signaling.

In summary, our study reveals RAC1 as a master regulator downstream of oncogenic MET to induce not only cell migration but also anchorage-independent growth (Figure 1). RAC1 classical role, which involved its GTPase activity, promoted by both PI3K isoforms p110-alpha and p110-beta, leads to cell migration, while RAC1 acts as a GTPase-independent adaptor, promoting mTOR localization at the plasma membrane, required for anchorage-independent growth. Further work is required to better understand oncogenic MET-RAC1-mTOR spatial signaling and how it is regulating cell growth. It would be interesting to investigate whether this signaling mechanism is used by other RTKs than MET. This discovery also suggests that a combinatorial treatment inhibiting both mTOR and PI3K provides the advantage of altering both tumor growth and metastasis, opening future avenues for MET/RTK driven cancer's therapies.

Disclosure of potential conflicts of interest

A.H. is an employee of The Institute of Cancer Research (ICR), which has a commercial interest in the discovery and development of PI3K inhibitors, including GDC0941 (Pictilisib), and operates a rewards-to inventors scheme. S.K. declares no conflict of interest.

Funding

A.H. was a recipient of a CR-UK studentship [C236/A11795], a Bayer4Target grant, and an ICR-funded postdoctoral fellowship. S. K. was a recipient of an MRC Project grant [MR/R009732/1].

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