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Deciphering the Signalling Complexity of Oncogenic Ras

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Commentary

Cancer cell signalling is complex and involves multiple interacting signals that concertedly drive disease progression in ways we do not understand. These signalling interactions occur not only in transformed cells but also between diverse cells in the tumour mass, leading to more aggressive and harder to treat cancers [1-7]. Fundamental questions about how these signalling events are initiated and integrated in cancer cells to achieve these effects remain largely unanswered. Addressing these questions will not only broaden our understanding of cancer biology but It also has the potential of highlighting signalling vulnerability that can be leveraged for designing effective therapies.

Oncogenic Ras mutations are prevalent in cancers and give rise to aggressive cancers *via* an intricate signalling network [8,9]. Animal models have been instrumental in dissecting signalling dynamics in development and diseases. Genetic studies in fruit flies and worms led to the characterization of the evolutionarily conserved Ras mitogen activated protein kinases (Ras/MAPK) signalling cascade during normal development [10-17]. Interestingly, targeting components of the Ras/MAPK signalling cascade in oncogenic Ras cancer cells only shows a modest suppressive effect on overgrowth, indicating that oncogenic Ras signalling does not merely amplify RAS/MAPK signals but rather it elicits signalling modalities that are distinct from normal Ras signalling.

We have been using a Drosophila tumour model to better understand the molecular underpinnings of oncogenic Ras-mediated tumour overgrowth and metastasis. This effort has led to the unexpected discovery of cell-intrinsic as well as cell-cell signalling interactions [2,7,18,19]. In [18], we address two long standing and puzzling observations highlighting the complexity of oncogenic Ras signalling: on the one hand, oncogenic Ras signalling requires its upstream receptor, Epidermal Growth Factor Receptor (EGFR) to exert its cancer promoting effect cancers [20-23], which could not be explained with our understanding of canonical EGFR/Ras signalling. How can the action of an activated molecule require the function of its upstream receptor? On the other hand, EGFR/Ras and Hedgehog (Hh) signalling are co-activated and cooperate in cancers [24-27]. Molecular mechanisms explaining why EGFR is required or how Hh is activated to cooperate with oncogenic Ras were unclear. Excitingly, we found that oncogenic Ras stimulates the transcription and secretion of EGF to recruit EGFR function. Surprisingly, rather than signalling *via* the known canonical EGFR signalling pathway, EGFR acts *via* the small G-protein and vesicle trafficking regulator ADP-Ribosylation Factor 6 (ARF6). ARF6 routes and stabilizes Hh on signalling competent endosomes to protect Hh from entering the degradation pathway. This ARF6-dependent control of Hh trafficking triggers Hh activation, which in turn synergizes with oncogenic Ras signalling to cause robust tumour overgrowth. Consistent with this mechanism, inhibition of EGFR or ARF6 causes Hh protein to be routed to the degradation pathway. In addition, depleting EGF or blocking EGFR function suppresses oncogenic Ras tumour overgrowth. More importantly, ARF6 or Hh knockdown prevents oncogenic Ras-mediated overgrowth in both fly and human cells. Notably, partial reduction of Hh protein levels was sufficient to significantly suppress oncogenic Ras-mediated tumour overgrowth, arguing for synergy between Hh and oncogenic Ras signalling. Taken together, this work provides the first unifying mechanism that explains both, the surprising role for EGFR in oncogenic Ras-mediated overgrowth and the oncogenic cooperation between EGFR/Ras and Hh signalling.

EGFR's role in oncogenic Ras signalling is likely tissue and/or context-dependent because its inhibition shows variable effects in different cancer types [20,23,28]. Oncogenic Ras signalling recruitment of EGFR function is intriguing not only because it defies our previous understanding of the EGFR/Ras signalling cascade but more importantly because it represents a mechanism for oncogenic Ras to engage ARF6 and control the cellular trafficking of pro-growth ligands in order to facilitate oncogenic synergy. EGFR's regulation of ARF6 likely affects the cellular trafficking of several other ligands and thus impacts multiple pathways. Consistent with this, ARF6 also controls the cellular transport of β-catenin and of the G protein subunit α q (GNAQ) to drive melanomas [29]. Therefore, ARF6 represents a molecular switch for the activation of diverse signals, which in turn cooperate with oncogenic Ras to promote tumourigenesis. Consequently, ARF6-targeted therapies offer a simpler strategy for modulating several cancer-relevant pathways all at once.

In addition to intracellular transport, oncogenic Ras signalling controls the secretion machinery to cell-intrinsically suppress tumour cell apoptosis and to elicit tumour-host cells signalling interactions that accelerate tumour overgrowth. We previously showed that on the one hand oncogenic Ras stimulates the secretion machinery to facilitate cellular clearance of the apoptotic ligand Tumour Necrosis Factor (TNF) and thus avert apoptosis. On the other hand, tumour-derived TNF triggers JNK (Janus NH2-terminal Kinase) signalling in the surrounding wild-type cells, which in turn stimulates the secretion of JAK/STAT (Janus kinase/Signal transducers and activators of transcription) ligands in the tumour milieu. This leads to the activation of JAK-STAT signalling in Ras tumour cells and results in robust tumour overgrowth2. The secretion machinery is conserved and its

deregulation has been implicated in various human cancers types, including Ras cancers [30-34], suggesting a broadly relevant mechanism for promoting cancer progression and highlighting a potential opportunity for therapy strategies.

In summary, together with our previous work, the focus study demonstrates that oncogenic Ras signalling achieves its characteristic complexity partly by controlling intracellular trafficking and cellular secretion. More broadly, the above studies collectively illustrate how one oncogenic mutation can, independent of any additional genetic lesions, trigger and incorporate other oncogenic signals in cancer cells. This has implications for how gene mutational status data are interpreted in cancer diagnostics or in cancer molecular characterization studies.

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