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AUTHOR'S VIEWS

All over the place: deciphering HRAS signaling from different subcellular compartments

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ABSTRACT

RAS (rat sarcoma virus oncogene homolog) oncogenes regulate fundamental biological processes through an ever-expanding signaling network. Using interaction proteomics, phosphoproteomics, transcriptomics, and integration of these datasets with a novel biostatistics approach, we have investigated Harvey-RAS (HRAS) signaling from different subcellular sites. The results reveal highly diversified signaling networks that regulate different aspects of HRAS functions.

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Auto-commentary

Despite more than 30 y of research, *RAS* (rat sarcoma virus oncogene homolog) is still one of the grand challenges in oncology. *RAS* oncogenes cause >30% of all human cancers, and *RAS* mutated tumors are generally refractory to currently available therapies.[1] RAS does not offer the traditional structural pockets where drugs can slip in, and targeting downstream effectors has proven equally elusive. RAS proteins cycle between inactive GDP-bound and active GTP bound states. Oncogenic mutations lock RAS into the active GTP-bound state, in which they can bind and activate many effector proteins that regulate all aspects of cellular physiology including proliferation, survival, metabolism, motility, adhesion, differentiation, and transformation. This large number of effector pathways makes it difficult to block RAS signaling by targeting downstream processes, and cognate efforts have been frustrating. An additional complication is that RAS can activate different signaling pathways from different subcellular compartments.[2] This complication however also opens a new conceptual approach to interfere with oncogenic RAS signaling in a highly targeted way by specifically blocking pathways that emanate from certain subcellular compartments. Although initial attempts to block RAS membrane localization by inhibiting its posttranslational processing did not yield clinically successful drugs, more recent efforts targeting proteins that mediate RAS localization to the plasma membrane appear promising.[1] However, to fully exploit this approach of targeting RAS by targeting its intracellular localization and avoid pitfalls, we need to understand how mutated RAS signals from different subcellular compartments and which processes are regulated in a localization specific manner. As biochemical processes can show unexpected behaviors that emerge from the properties of the network context rather than the properties of individual proteins,[3] it is important to perform this analysis on a global network level.

In order to do this we have investigated Harvey-RAS (HRAS), signaling from four different subcellular localizations performing interaction proteomics, phosphoproteomics, and transcriptomics and integrating these data into coherent networks that allow to globally analyze HRAS signaling (Figure 1).[4] In order to determine the mutant HRAS interactome, oncogenic mutant HRASV12 was equipped with "zip code-tags" that targeted it to the disordered plasma membrane (DM), plasma membrane lipid rafts (LR), the Golgi apparatus (GA), or the endoplasmic reticulum (ER), and HRASV12 binding proteins were identified by quantitative mass spectrometry. Unmodified HRASV12 was used as a control. Out of 397 interactors, only 21 were shared between all localizations indicating a high degree of site-specific diversity. To analyze downstream signaling we mapped the phosphoproteome regulated by "zip-coding" an HRAS specific guanine nucleotide exchange factor (GEF) domain. In this way, only endogenous HRAS present at these different sites becomes activated. Again, very few phosphorylation events were shared, but the vast majority was regulated by HRAS activated at the DM and LR. We also devised a statistical method to identify substrates and their cognate kinases allowing us to analyze the phosphorylation of transcription factors (TFs) in detail. Most TFs were phosphorylated by 1–2 kinases, but v-myc avian myelocytomatosis viral oncogene homolog (MYC), retinoblastoma 1 (RB1), and signal transducer and activator of transcription 3 (STAT3) were targeted by 15–20 kinases, all regulated from the plasma membrane (PM). Notably, these TFs are intimately involved in cancer, with *MYC* and *STAT3* being oncogenes

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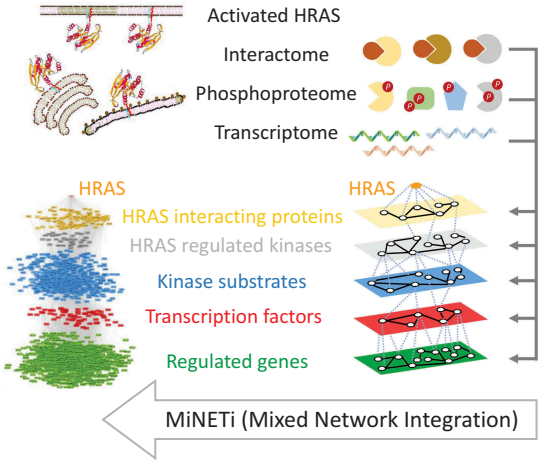

Figure 1. Integrative analysis of compartmentalized HRAS signaling. Site-specific Harvey-RAS (HRAS) interaction proteomics, phosphoproteomics, and transcriptomics data were collected and converted into networks. Nodes in the networks were linked by logical and empirical rules, e.g. kinases have substrates and usually, they physically associate, within a statistical framework termed mixed network integration (MiNETi). The resulting integrated network allows to analyze signal flow and regulation across different types of omics datasets. The figure is from Santra et al.[4]

and *RB1* a tumor suppressor. Thus, activated HRAS uses these key TFs as integration points for signals originating from the PM. At the transcriptional level, most genes were regulated in a site-specific manner with twice as many genes regulated from the ER than from all other localizations combined. Although the TFs regulated by HRAS from different sites were largely the same, they could regulate many more genes when controlled from the ER, suggesting that the transcriptional diversification of HRAS signaling is due to different posttranslational modifications of the same TFs rather than the use of different TFs.

Having three global biochemical networks we developed MiNETi (mixed network integration) to enable an integrated global analysis of HRAS signalling.[4] While we have made great progress in the analysis of individual types of omics datasets, integrating them into one coherent view remains a grand challenge. To the best of our knowledge, no universal framework exists that allows the integration of several different heterogenous datasets. MiNETi uses a simple concept that permits the integration of any type of data that can be displayed as a network. Nodes in the different networks are linked up by logical and empirical rules, e.g. some of the HRAS binding proteins are kinases, which have substrates that include TFs which regulate genes. Thus, links are intuitive, informative and make full use of existing knowledge. The method scales easily as new networks can be added or intercalated. Importantly, as rules can be statistically weighted, MiNETi can also make quantitative statements. We are currently extending MiNETi so that rules can be optimized and learned using machine learning techniques.

The power of MiNETi was demonstrated by the fact that we could identify most of the known HRAS signaling pathways, and discover new aspects of HRAS signaling, e.g. that HRAS regulates migration from the ER and cell survival via the tumor protein p53 (TP53) tumor suppressor from the GA. In broader terms, HRAS signaling from the PM seems to collect few inputs, process them extensively in cytosolic signaling pathways and integrate them in the nucleus by regulating a small set of TFs and genes. When signaling from endomembranes, HRAS processes many inputs via a small number of signaling pathways to control many genes. This extensive dataset and MiNETi data integration framework enable a deep analysis of compartmentalized HRAS signaling.

Disclosure of potential conflicts of interest

There are no potential conflicts of interest to disclose.

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