Understanding and drugging RAS: 40 years to break the tip of the iceberg

Donita C. Brady1,2,*, Julija Hmeljak3 and Arvin C. Dar4,*

ABSTRACT
Several cancers and rare genetic diseases are caused by dysregulation in the RAS signaling pathway. RAS proteins serve as molecular switches that regulate pathways involved in cellular growth, differentiation and survival. These pathways have been an intense area of investigation for four decades, since the initial identification of somatic RAS mutations linked to human cancers. In the past few years, inhibitors against several RAS effectors, as well as direct inhibitors of the K-RAS mutant G12C, have been developed. This Special Issue in DMM includes original Research articles on RAS-driven cancers and RASopathies. The articles provide insights into mechanisms and biomarkers, and evaluate therapeutic targets. Several articles also present new disease models, whereas others describe technologies or approaches to evaluate the function of RAS in vivo. The collection also includes a series of Review articles on RAS biology and translational aspects of defining and treating RAS-driven diseases. In this Editorial, we summarize this collection and discuss the potential impact of the articles within this evolving area of research. We also identify areas of growth and possible future developments.

KEY WORDS: Cancer, Developmental disorders, RAS inhibitor, RAS pathway

Introduction
The RAS superfamily of small GTPases includes over 150 members in humans (Wennerberg et al., 2005). These proteins generally function as molecular switches and regulators of cellular communication, linking cues from the cell surface to changes in gene expression and protein translation (Pylayeva-Gupta et al., 2011). The four RAS oncoproteins, K-RAS4A, K-RAS4B, N-RAS and H-RAS, encoded by three RAS genes, are the founding members of the family and recognized for their critical roles in cancer (Moore et al., 2020). Indeed, one in four human cancers contain mutant forms of RAS, with substitutions frequently observed in pancreatic cancer (~88% K-RAS mutation positive), colon cancer (~50% K-RAS), lung cancer (~32% K-RAS) and melanoma (~17% N-RAS) (Prior et al., 2020). These key members, together with additional RAS homologs, have been implicated in other diseases, including common Mendelian disorders referred to as RASopathies (Simanshu et al., 2017), non-neoplastic cerebral diseases, such as Alzheimer’s and Parkinson’s disease (Qu et al., 2019), and spinocerebellar ataxia type 1 (Park et al., 2013).

2022 marks the 40th anniversary of the initial discovery of RAS mutations in human cancers (Der et al., 1982; Parada et al., 1982; Santos et al., 1982). Over this time, RAS family members – in particular K-RAS due to the prevalence of the mutants – have been intensively studied and have captivated scientists from a range of disciplines, including structural biology, biochemistry, chemistry, signal transduction, model organism development, genomics and
The story of RAS from human genetics through to the first direct inhibitors represents a long and winding road of discovery, which has been greatly accelerated by a focused community of academic and industry scientists (Nissley and McCormick, 2022). However, we are likely only at the tip of the iceberg for understanding RAS (Fig. 1). Under the surface, much remains to be understood about different RAS family members and about targeting the dysregulated pathways with therapeutic interventions. This Special Issue of Disease Models & Mechanisms (DMM) was thus conceived to collate cutting-edge research on the roles of RAS in cancer and developmental disorders, and on approaches to treat and modify the disease course in model systems. In this Editorial, we reflect on this Special Issue and how its articles fit into the broader context of this exciting field.

The RASopathies
Germline mutations in genes encoding components of the RAS pathway cause a group of rare developmental disorders called the RASopathies. Improved understanding of the mechanisms of RAS dysregulation, coupled with expanded clinical sequencing means that the definition of RASopathy continues to evolve, as discussed in Katherine Rauen’s Perspective (Rauen, 2022). This article explores the enigma of defining a RASopathy through the lens of oncologists, medical geneticists, RAS biologists, and patients and their families. This holistic approach aims to advance our understanding of these developmental disorders and how they can be diagnosed and treated. Research in RAS dysregulation, both in the context of cancer and of RASopathies, has identified several potential therapies for RASopathy patients, as discussed in the Special article by Corina Anastasaki and David Gutmann (Anastasaki and Gutmann, 2022) and in the Review from Marielle Yohe and team (Hebron et al., 2022). Indeed, a research article from Katherine Rauen’s group describes a newly developed murine model of the RASopathy Costello syndrome and demonstrates that MEK inhibition is an effective strategy in mitigating the muscular damage caused by aberrant H-RAS (Tidyman et al., 2021).

Whilst RASopathies are developmental disorders driven by mutations in components of the RAS pathway (Rauen, 2022), the involvement of aberrant RAS signaling in development reaches beyond this group of disorders. Toshihiro Inubushi and colleagues found that a transcriptional regulation pathway downstream of RAS is required in normal palatogenesis and its perturbation may be involved in cleft palate (Inubushi et al., 2022). A new mouse model was also instrumental in understanding the role of sensory neuron genesis and differentiation in the neurodevelopmental disorder DiGeorge (also known as 22q11.2 deletion) syndrome (Karpinski et al., 2021), which is caused by broad transcriptional deregulation affecting RAS signaling among other crucial pathways. Taken together, the RASopathies-focused articles in this Special Issue highlight recent progress in the field, which is already bringing tangible benefits to patients.
Voices beyond this Special Issue

In this Special Issue, DMM editor Ross Cagan interviewed Kevan Shokat to discuss his success in ‘drugging the undruggable’ RAS and how blurring the boundaries between chemistry and biology can help develop novel cancer therapeutics (Cagan and Shokat, 2022). In her ‘A Model for Life’ interview, Shiva Malek talks about her career, personal attitudes towards mentorship, and how targeting RAS-driven cancers requires strong collaboration between academia and industry (Malek, 2021).

These conversations with leaders in the RAS field show how knowledge has evolved quickly in recent years, most notably with the introduction of novel drugs to enable translational studies. K-RASG12C was initially tractable due to the unique reactivity of the cysteine residue and the ability to find compounds that covalently target the mutant (Ostrem et al., 2013). Extending these approaches to other RAS mutants is currently underway, and it appears that the K-RASG12D mutant is also amenable to direct inhibition (Wang et al., 2021). In parallel, clinical studies have begun to report mechanisms of acquired resistance to K-RASG12C inhibition (Awad et al., 2021; Tanaka et al., 2021; Zhao et al., 2021). Similarly to studies on the rapid emergence of resistance against BRAFV600E inhibitors (Swanton et al., 2014), this work highlights the inherent challenges of targeting single alleles. Common mechanisms to evade inhibitors, including mutations directly in the drug-binding pocket, overexpression of the oncogene and alterations in various effector pathways, mean that successfully targeting aberrant RAS signaling is an ongoing task. There have been several combination strategies with inhibitors of targets that function upstream, like SHP2 (PTPN11) (Fedele et al., 2021; Ryan et al., 2020) and EGFR (Amidio et al., 2020; Xue et al., 2020), or downstream of RAS (Adachi et al., 2020; Lou et al., 2019; Misale et al., 2019; Molina-Arcas et al., 2019; Santana-Codina et al., 2020). Additionally, combinations based on targeting immune-modulatory pathways (Briere et al., 2021; Canon et al., 2019) are under investigation. These efforts hope to expand the number of patients that benefit from direct KRAS inhibitors, as well as extend the duration of responses.

In addition to the translational work, recent years have brought further insight into the basic biology of RAS. For example, researchers have made major advances in our understanding of the structural biology of complexes, drug-binding mechanisms, and the regulation of distinct target activation states (Kondo et al., 2019; Liao et al., 2020; Martinez Fiesco et al., 2022; Park et al., 2019). Moreover, fundamental studies on how various mutants operate and why there could be strong lineage dependence for individual alleles is another exciting area of research. We anticipate that such insights focused on understanding RAS biology in cancer and RASopathies will have broad implications for many classes of diseases. Along such lines, targeting of the RAS pathway in diseases of aging (Fabian et al., 2021; Slack et al., 2015) and neurological disorders (Park et al., 2013; Qu et al., 2019) with anti-cancer therapeutics has already demonstrated promise for advances in these areas.

Conclusions

The articles in and beyond this Special Issue highlight significant advances in the field, from improved understanding of aberrant RAS signaling to therapy development. The increasing amount and validity of novel cell and animal models, even for some of the rarest RASopathies and RAS-driven cancers, will allow for further characterization of the disease pathology and can facilitate the development of novel therapeutic interventions. Recognizing the overlap between RASopathies, aging, neurological disorders and RAS-driven cancer shows how enhancing knowledge of each of these diseases can be mutually beneficial. It has also become clear that standardizing outcome measures and validating findings are of utmost importance for effective translation from model systems to patients. This rigor can propel the RAS field forward and result in better selection of candidate compounds and their most effective combination strategies to target multiple levels of the signaling pathway, eventually leading to a higher success rate in clinical trials for the broad range of diseases that are linked to RAS. We hope that you enjoy this Special Issue and the ongoing subject collection, which will continue to grow as new articles on the topic are published in DMM.

This article is part of a collection ‘The RAS Pathway: Diseases, Therapeutics and Beyond’, which was launched in a dedicated Special Issue guest edited by Donita Brady and Arvin Dar. See related articles in this collection at https://journals.biologists.com/dmm/collection/5089/The-RAS-Pathway.

Acknowledgements

We thank DMM’s authors, reviewers and editors who helped to compile this Special Issue and ongoing subject collection. Particular thanks to Monica Justice and Liz Patton for handling many of the research articles published in this issue, and to Ross Cagan for his insightful conversation with Kevan Shokat.

Funding

A.C.D. gratefully receives funding from the National Institutes of Health (R01 CA227763, R01 CA258736, R01 CA256480, R56 AG066712 and P30 CA196521) and the Mark Foundation for Cancer Research. A.C.D. also thanks the Pershing Square Sohn Cancer Research Alliance and Alex’s Lemonade Stand Foundation for Childhood Cancer for support. D.C.B. gratefully receives funding from the National Institutes of Health (R53 GM124749) and the Ludwig Cancer Center Princeton Branch.

References

Adachi, Y., Ito, K., Hayashi, Y., Kimura, R., Tan, T. Z., Yamaguchi, R. and Ebi, H. (2020). Epithelial-to-mesenchymal transition is a cause of both intrinsic and acquired resistance to KRAS G12C inhibitor in KRAS G12C–mutant non–small cell lung cancer. Clin. Cancer Res. 26, 5962-5973. doi:10.1158/1078-0432.CCR-20-2077
Ah Pai, M. A. and Abblain, J. (2022). RAS pathway regulation in melanoma. Dis. Model. Mech. 15, dmm049229. doi:10.1242/dmm.049229
Amidio, V., Yaeger, R., Arcella, P., Cancelleri, C., Lamba, S., Lorenzato, A., Arena, S., Montone, M., Mussolin, B., Bian, Y. et al. (2020). EGFR blockade reverts resistance to KRASG12C inhibition in colorectal cancer. Cancer Discov. 10, 1129-1139. doi:10.1158/2159-8290.CD-20-0187
Anastasaki, C., Orozco, P. and Gutmann, D. H. (2022). RAS and beyond: the many faces of the neurofibrinomatosis type 1 protein. Dis. Model. Mech. 15, dmm049362. doi:10.1242/dmm.049362
Awad, M. M., Liu, S., Rybinsk, I. I., Arbour, K. C., Dilly, J., Zhu, V. W., Johnson, M. L., Heist, R. S., Patil, T., Riely, G. J. et al. (2021). Acquired resistance to KRASG12C inhibition in cancer. N. Engl. J. Med. 384, 2382-2393. doi:10.1056/NEJMoa2105281
Bangs, F. K., Miller, P. and O’Neill, E. (2020). Ciliogenesis and Hedgehog signalling are suppressed downstream of KRAS during acinar-ductal metaplasia in mouse. Dis. Model. Mech. 13, dmm044289. doi:10.1242/dmm.044289
Briere, D. M., Li, S., Rybinski, I. I., Arbour, C., Dilly, J., Zhu, V. W., Johnson, M. L., Heist, R. S., Patil, T., Riely, G. J. et al. (2021). Acquired resistance to KRASG12C inhibition in cancer. Nature 5962-5973. doi:10.1158/1078-0432.CCR-20-2077
Cagan, R. and Shokat, K. (2022). Drugging the undruggable: Ross Cagan interviews Kevan Shokat. Dis. Model. Mech. 15, dmm049468. doi:10.1242/dmm.049468
Canon, J., Rex, K., Saiki, A. Y., Mohr, C., Cooke, K., Bagal, D., Gaida, K., Holt, T., Knutson, C. G., Koppada, N. et al. (2019). The clinical KRAS(G12C) inhibitor AMG 510 drives anti-tumour immunity. Nature 575, 217-223. doi:10.1038/s41586-019-1694-1
Denes, D. K., Fuentealba, M., Dieter, H. M., Partridge, L. and Thornton, J. M. (2021). Functional conservation in genes and pathways linking ageing and immunity. Immunity Ageing 18, 23. doi:10.1186/s12979-021-00232-1
DMM
Xue, J. Y., Zhao, Y., Aronowitz, J., Mai, T. T., Vides, A., Qeriqi, B., Kim, D., Li, C., de Stanchina, E., Mazutis, L. et al. (2020). Rapid non-uniform adaptation to conformation-specific KRAS(G12C) inhibition. *Nature* **577**, 421-425. doi:10.1038/s41586-019-1884-x

Zhao, Y., Murciano-Goroff, Y. R., Xue, J. Y., Ang, A., Lucas, J., Mai, T. T., da Cruz Paula, A. F., Saiki, A. Y., Mohn, D., Achanta, P. et al. (2021). Diverse alterations associated with resistance to KRAS(G12C) inhibition. *Nature* **599**, 679-683. doi:10.1038/s41586-021-04065-2

Zhu, J.-y., Huang, X., Fu, Y., Wang, Y., Zheng, P., Liu, Y. and Han, Z. (2021). Pharmacological or genetic inhibition of hypoxia signaling attenuates oncogenic RAS-induced cancer phenotypes. *Dis. Model. Mech.* **15**, dmm048953. doi:10.1242/dmm.048953