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Review

KRAS-Mutant non-small cell lung cancer: From biology to therapy

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ABSTRACT

In patients with non-small cell lung cancer (NSCLC), the most frequent oncogene driver mutation in Western countries is Kirsten rat sarcoma viral oncogene homolog (*KRAS*), and *KRAS*-mutant NSCLC is associated with smoking. There are various sources of biological heterogeneity of *KRAS*-mutant NSCLC, including different genotypes that may be associated with specific clinical outcomes, the presence of other co-mutations that exhibit different biological features and drug sensitivity patterns, and mutant allelic content. The efficacy of chemotherapy in patients with *KRAS*-mutant NSCLC is generally poor and numerous novel therapeutic strategies have been developed. These approaches include targeting *KRAS* membrane associations, targeting downstream signalling pathways, the use of *KRAS* synthetic lethality, direct targeting of *KRAS*, and immunotherapy. Of these, immunotherapy may be one of the most promising treatment approaches for patients with *KRAS*-mutant NSCLC. Recent data also suggest the potential for distinct efficacy of immunotherapy according to the presence of other co-mutations. In view of the biological heterogeneity of *KRAS*-mutant NSCLC, treatment will likely need to be individualised and, in future, may require the use of rational combinations of treatment, many of which are currently under investigation.

1. Introduction

Kirsten rat sarcoma viral oncogene homolog (*KRAS*) mutations are found in approximately 20–25% of lung adenocarcinomas in Western countries [1–3] and in approximately 10–15% of cases in Asia [4,5]. Considered globally, *KRAS*-mutant tumours constitute the most frequent potentially targetable molecular subtype of non-small-cell lung cancer (NSCLC) [6]. As is the case with the vast majority of potentially

Abbreviations: BATTLE, Biomarker-Integrated Approaches of Targeted Therapy for Lung Cancer Elimination; BET, bromodomains and extra-terminal domain; CDK, cyclin-dependent kinase; Chk1, checkpoint kinase 1; CI, confidence interval; CUSTOM, Molecular Profiling and Targeted Therapies in Advanced Thoracic Malignancies; DDR1, discoidin domain receptor 1; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; FGFR, fibroblast growth factor receptor; FOXO, forkhead box O; GAPs, GTPase activating proteins; GEFs, guanine nucleotide exchange factors; GGTase, geranylgeranyl transferase; HIF1α, hypoxia inducible factor-1 alpha; HR, hazard ratio; HRAS, Harvey rat sarcoma viral oncogene homolog; ICMT, isoprenyl carboxyl methyltransferase; IFN, interferon; IL, interleukin; JNK, Jun N-terminal kinase; JUNIPER, A Study of Abemaciclib (LY2835219) in Participants With Previously Treated KRAS Mutated Lung Cancer; KRAS, Kirsten rat sarcoma viral oncogene homolog; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; MISSION, Monotherapy admInistration of Sorafenib in patientS with nOn-small-cell luNg cancer; MK2, MAPK-activated protein kinase 2; mTOR, mammalian target of rapamycin; NF, nuclear factor; NRF2, nuclear factor (erythroid-derived 2)-like 2; NSCLC, non-small-cell lung cancer; OR, odds ratio; ORRs, objective response rates; OS, overall survival; PDK1, phosphoinositide-dependent kinase 1; PD-L1, ligand of programmed death-1 receptor-1; PFS, progression-free survival; PIP2, phosphatidylinositol 4,5-biphosphate; PIP3, phosphatidylinositol 3,4,5-biphosphate; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; RAS, rat sarcoma virus; RCE1, RAS-converting enzyme 1; RHOA, Ras homolog gene family member A; SELECT-1, Selumetinib Evaluation as Combination Therapy; shRNA, short hairpin RNA; siRNA, small interfering RNA; TAILOR, TArceva Italian Lung Optimization tRial; TKIs, tyrosine kinase inhibitors; TORCMEK, Study of AZD2014 in Combination With Selumetinib in Patients With Advanced Cancers; TTF-1, thyroid transcription factor-1

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actionable genetic alterations in NSCLC, *KRAS* mutations are almost exclusively detected in lung adenocarcinomas and are rarely found in squamous-cell cancers [7,8]. Unlike other oncogene-driven lung cancers that commonly arise in never or light smokers, *KRAS* mutations are typically found in tumours from patients who smoke (often heavy smokers), and only ≈5–10% of *KRAS*-mutant lung cancers arise in never or light smokers [1,2].

In the case of NSCLC, *KRAS* mutations occur predominantly (95%) at codons 12 (> 80%) and 13 [1]. The most frequent codon variant, accounting for approximately 39% of *KRAS*-mutant NSCLCs, is the *KRAS*-G12C mutation [1,2]. Other common mutations include *KRAS*-G12 V (18–21%) and *KRAS*-G12D (17–18%) variants [1,2]. Remarkably, smokers and never smokers have a different spectrum of mutations and codon variants in KRAS. Thus, transition mutations (G > A) are more frequent in never smokers, whereas transversion mutations (G > C or G > T) are more common in former or current smokers [1,9]. In addition, *KRAS*-mutant tumours from smokers are genomically more complex, with a higher mutational burden and higher frequency of major co-occurring mutations in *TP53* or *STK11* than tumours from never smokers [9].

From a clinical standpoint, *KRAS*-mutant lung cancers have generally been associated with poorer overall survival (OS) than *KRAS* wild-type tumours, particularly in the advanced-stage setting [10–12]. However, other studies in early-stage [3] or advanced-stage cohorts [13] have not been consistent in validating this poorer survival; thus, the prognostic significance of *KRAS* mutational status in lung cancer remains a topic of debate. Notably, growing evidence indicates that *KRAS*-mutant NSCLC might not be a unique entity, and recent translational studies have unravelled some of the main clinically relevant determinants of this biological diversity. These discoveries may have prognostic significance and may also impact on the benefit of different biological therapeutic strategies for this disease.

Although there is evidence that at least a subset of *KRAS*-mutant cancers are indeed KRAS dependent [14,15], developing molecularly driven therapeutics to directly or indirectly block KRAS activity has proven difficult, and no such therapeutic has demonstrated robust activity in the clinic. However, recent discoveries in KRAS biology, together with the advent of immune therapies, might result in the development of effective treatment strategies and optimal therapeutic stratification of *KRAS*-mutant NSCLC in the near future.

In this article, we briefly summarise some clinically relevant aspects of the biology and biological heterogeneity of *KRAS*-mutant NSCLC and provide an update on molecularly driven therapies for this disease. We especially emphasise the role of immune checkpoint blockade in this particular molecular subtype of lung cancer.

2. Biology of *KRAS*

2.1. *KRAS* function and major downstream effector pathways

KRAS, a member of the human *RAS* gene family, encodes a small GTPase membrane-bound protein, which can exist in two different states: GDP bound, which is inactive, and GTP bound, which is active and transduces signals by interacting with different downstream effectors. RAS proteins act as a cellular switch that is turned on by extracellular stimuli, resulting in the transient formation of the active, GTP-bound form of RAS, which activates different signalling pathways that regulate fundamental cell processes [16,17]. The activation of RAS signalling is regulated by the regulator factors that produce the GDP–GTP exchange (guanine nucleotide exchange factors; GEFs) or affect its GTPase activity (GTPase activating proteins; GAPs). GEFs enhance GDP release from RAS and permit its replacement with GTP, resulting in RAS activation. GAPs increase the intrinsic GTPase activity of RAS, resulting in rapid conversion from the active to inactive GDP-bound RAS [18,19]. Mutated RAS oncoproteins differ functionally from their normal counterparts [20–22] in that the oncogenic forms prevent GAP from increasing the intrinsic catalytic rate of GTPase, thereby keeping RAS in its constitutively GTP-bound active state, which activates oncogenic pathways and cellular signal transduction [23,24].

In addition to GTP binding, RAS proteins must associate with cellular membranes, where they interact with GEFs and other upstream regulators, in order to transmit extracellular signals downstream. RAS proteins are synthesised as soluble precursors, which then have to be post-translationally modified to mediate their association with membranes [25–27]. The enzymes that catalyse these modifications are also interesting targets for the development of anti-RAS therapies.

The signalling network that RAS regulates defines the biological effects of RAS. For this reason, it is so important not only to understand how RAS is activated but also to know its downstream molecular effectors. RAS proteins have a central role in transducing mitogenic signals, so their main downstream effectors are involved in the activation of mitogenic pathways, which are implicated in tumourigenesis. There are more than ten reported effectors of RAS, and below we describe those that have more importance in RAS-mediated tumourigenesis [17,28] (Fig. 1).

RAF is the first kinase in the mitogen-activated protein kinase (MAPK) pathway, which phosphorylates MEK, which in turn activates the extracellular signal-regulated kinase (ERK). ERK both activates cytosolic substrates and translocates to the nucleus to stimulate the expression of diverse genes involved in cell proliferation, survival, differentiation and cell-cycle regulation. It has been widely demonstrated that the MAPK signalling pathway plays an essential role in RAS-mediated tumourigenesis [29–31] (Fig. 1).

Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) also plays a key role in RAS-mediated tumourigenesis [32,33]. When activated, PI3K converts phosphatidylinositol 4,5-biphosphate (PIP2) to phosphatidylinositol 3,4,5-triphosphate (PIP3) by phosphorylation, and PIP3 activates phosphoinositide-dependent kinase 1 (PDK1), which in turn phosphorylates AKT, a serine/threonine-specific protein kinase. AKT activation leads to phosphorylation of its several physiological substrates such as mammalian target of rapamycin (mTOR), forkhead box O (FOXO) or nuclear factor (NF)-κB, which stimulate cell-cycle progression, survival, metabolism, and migration and resistance to apoptosis.

The RALGDS is another RAS effector [34], whose substrates are the RAS family RAL-A/B small GTPases. RALGDS can also signal through the Jun N-terminal kinase (JNK) pathway to stimulate transcription of pro-survival and cell-cycle progression genes. This signalling pathway also has a very significant role in RAS-mediated oncogenesis [35–37].

In summary, RAS proteins regulate, through different effectors, signal transduction that controls diverse cellular functions, such as survival, proliferation and differentiation. The constitutively activated RAS oncoproteins initiate intracellular cascades without the existence of extracellular signals, resulting in uncontrolled cell proliferation and abnormal cell survival. Deregulation of these cellular functions constitutes many of the hallmarks of cancer [38].

2.2. Biological heterogeneity of KRAS-mutant NSCLC

Accumulating evidence suggests that *KRAS*-mutant lung cancers might be composed of a heterogeneous set of distinct diseases. At least three main relevant drivers of this biological diversity may have direct clinical implications: 1) the presence of co-occurring genetic events, 2) the different *KRAS* mutation subtypes, and 3) the mutant *KRAS* allelic content.

Unlike other oncogene-driven lung cancers, *KRAS*-mutant lung tumours frequently appear with other major genetic co-mutations. By using RNAseq analysis in cohorts of early- and advanced-stage *KRAS*-mutant tumours, Skoulidis et al. identified three robust expression-based clusters that were dominated by the presence of *STK11* co-mutations (KL subgroup), *TP53* co-mutations (KP subgroup) and *CDKN2A/B* inactivation plus low thyroid transcription factor-1 (TTF-1)