Editorial

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Future Medicinal Chemistry

KRAS^{G12C} inhibitors on the horizon

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RAS proteins (the four isoforms KRAS4A, KRAS4B, NRAS and HRAS encoded by three genes *KRAS, NRAS* and *HRAS*) act as molecular switches that when activated drive several key cellular processes such as cell growth, proliferation and survival [1]. In normal cells, RAS activity is under tight control by the precise activation (binding to GTP) and inactivation (GTP hydrolysis to GDP) [1]. As with other critical proteins, it is not at all surprising to note that the gene encoding the RAS protein isoforms is found mutated or altered in a significant proportion of tumors [2]. Mutant RAS loses its ability to hydrolyze GTP and remains in a permanently activated state (bound to GTP) leading to uncontrolled growth. These facts have been known for more than 30 years and considerable pharmaceutical investments have been directed to develop inhibitors of RAS, its activators or downstream effectors [3]. Nevertheless, until this day there has been no effective therapy developed that could block RAS signaling. Direct targeting of RAS has remained challenging. The most simplistic approach to target any protein is to identify pockets in its structure where a small molecule type drug can bind. However, the only available binding site in KRAS is the GTP-binding pocket where the catalysis of GTP–GDP occurs. GTP almost exclusively occupies this site with extremely high affinity (that falls in the picomolar range) making the development of a competing small molecule an improbable task. Similarly, targets within the RAS pathway (upstream or downstream) have also turned out to be not very effectual clinically. These hurdles have led researchers to believe that the RAS fortress is impenetrable [4].

In recent years, KRAS mutation specific targeted approaches have gained steam within the RAS research community [5]. These efforts are coming from independent academic research labs, pharmaceutical industry and also through the NCI RAS initiative [6]. Among the different known mutations, KRAS^{G12C} (glycine 12 to cysteine) is quite prevalent and has been considered druggable. KRAS^{G12C} mutations are frequently observed in non-small-cell lung cancer (NSCLC; ~40%) although rarely in pancreatic ductal adenocarcinoma (which carries ~50–80% KRAS^{G12D} mutations). Aptly termed as a covalent handle, the cysteine residue is amenable to covalent targeting by small molecule type compounds. Studies have shown that due in part to the close proximity of cysteine 12 to both the nucleotide pocket and the switch regions, thiol reactive compounds can bind to the active site covalently and inhibit KRAS^{G12C} mutation driven signaling [7]. Several labs have proposed the cysteine thiol of G12C mutant KRAS as a target and have used a variety of disulfide or Michael acceptor containing thiol reactive small compounds to inhibit KRAS^{G12C}. The lack of cysteine moiety in wild-type KRAS would give a degree of selectivity to these compounds.

The feasibility of such an approach was proven by Ostrem *et al.*, as they designed strategies to keep KRAS in a GDP-bound state by using thiol-reactive compounds [8]. Their approach focused on targeting the nucleotide-dependent SWITCH II region that is conformational reliant regulator of RAS signaling. Their thiol-reactive drugs were shown to create structural modification (allosteric site) in the SWITCH II region resulting in inactivation of KRAS (GDP-bound state).

Other approaches in this direction include the development of thiol-reactive GDP analogs to inactivate KRAS in a way that competes with nucleotide binding. Developed by Westover and colleagues, such compounds were shown to keep KRAS in a permanently inactive (GDP-bound) type state in cell line models [9]. In principle, these types of

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compounds focus on targeting the $KRAS^{G12C}$ in the inactive GDP-bound state. In another study it is also shown that another thiol-reactive inhibitor ARS-853 can selectively bind to GDP-bound $KRAS^{G12C}$ but not GTP-bound state [10]. However, given that majority of the RAS is GTP bound, there will be limitations into how much target is accessible/available. ARS-1620 is yet another small molecule type drug that binds covalently to $KRAS^{G12C}$ with high selectivity and potency [11]. ARS-1620 was shown to possess sufficient inhibitory effects on KRAS signaling and causes proliferation inhibition *in vitro* and tumor suppression in patient derived *in vivo* models.

These proof-of-concept compounds give significant traction to the concept of directly targeting $KRAS^{G12C}$ to tame mutant RAS. On a cautionary note, like other small molecule strategies against cancer targets such as EGFR, the development of resistance may be an issue to deal with. However, in this case the major advantage is that cysteine in $KRAS^{G12C}$ is both the small molecule attachment site as well as the activation mutation. This means that for resistance development, the cysteine needs to be replaced by another similar activating mutation. The possibility for such unique activation mutation to occur is quite rare. There are several additional challenges associated with the thiol-targeted drugs. For example, Mitchell *et al*. showed that under physiological conditions there is a predominant existence of the $KRAS^{G12C}$ thiol in thiolate (-S) state [12]. The thiolates are more readily oxidized and this can induce conformational changes that can abrogate the inhibitory effects of $KRAS^{G12C}$ specific small molecule drugs. Whether such oxidation state of thiol group can be harnessed to develop unique class of redox-sensitive small molecule drugs remains to be seen (for further reading, see National Cancer Institute RAS blog [13]).

There has been considerable dollar investment in the area of small molecule drug development that could interfere with RAS signaling. However, these attempts have met significant hurdles that have hindered the development of potent small molecule type drugs that could significantly block RAS function, its activators and downstream effectors. These persistent failures call for out-of-the-box ideas and fresh new tools to facilitate drug development against such an unmet clinical need. The emergence of $KRAS^{G12C}$ inhibitors that target the cysteine handle is a significant and positive development. Simultaneously, several novel research tools (screening assays, cell lines and animal models) have also been developed by the RAS research community. Among these, the RAS-less MEFs, developed through the RAS Initiative at the Frederick National Laboratory for Cancer Research labs [14] need to be especially mentioned. These are important tools that are anticipated to further aid in the development of mutation-specific RAS inhibitors. Such work brings new motivation to target $KRAS^{G12C}$ as a unique and druggable avenue within the by far unconquerable RAS signaling.

Acknowledgments

Part of this editorial highlights available knowledge on $KRAS^{G12C}$ drugs described in the National Cancer Institute RAS blog [12] entitled "Mutation-Specific Approaches to KRAS Cancers: What We Can Learn from G12C-Directed Inhibitors" that was originally published by the National Cancer Institute.

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