Small Molecule Inhibition of Oncogenic KRAS and Downstream Signaling Pathways in Pancreatic Ductal Adenocarcinoma

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***Abstract*— Common recurring mutations in certain cell signaling pathways continue to be found in the progression and metastasis of Pancreatic ductal Adenocarcinoma (PDAC), with Ki-ras2 Kirsten rat sarcoma viral oncogene (KRAS), a family of genes controlling control cell growth, being the most frequent one. Understanding the nuances of this mutation and its downstream effectors can provide a blueprint for effective treatment methods using small molecule inhibitors. This review will elucidate how activation of the mutated KRAS protein leads to the eventual activation of various intracellular pathways, leading to cell proliferation. In addition, new targeted therapeutic treatments in the form of small molecule inhibitors will also be discussed.**

I. Introduction

With late stage diagnosis and unsuccessful treatment methods, Pancreatic ductal adenocarcinoma (PDAC) is on the road to become the second most common cause of cancer-related deaths by 2030 [1]. Over the past 10 years, new therapeutic approaches have emerged to increase the survival rate of patients with PDAC, but lack of early diagnosis causes prognosis to remain disappointing. Identifying the type of mutated cell signaling pathway in the patient and using targeted therapy may solve this problem. The KRAS isoform of Ras is a commonly mutated oncogene, a gene that due to stressors can turn into a tumor cell. KRAS plays a major role in the progression and invasion of PDAC tumors, and its constitutive activation is found in over 90% of PDAC cases [2]. The Mutational KRAS protein is permanently attached to GTP (Guanosine triphosphate) and continually activates downstream signaling pathways such as nuclear factor κB (NF-κB), phosphoinositide 3-kinase (PI3K), JUN N-terminal kinase (JNK), and the Mitogen-activated protein kinase (MAPK) pathway, which all lead to tumorigenesis, the formation of tumors [3].

II. Inhibition of KRAS

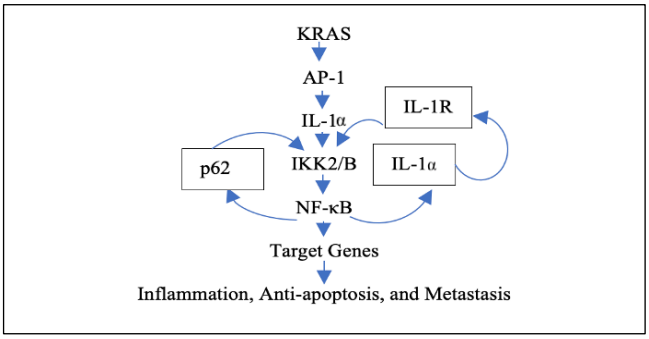
The role of KRAS has been studied in detail over the past few decades as it is a key marker for PDAC prognosis. However, due to its constant activation of GTP and its reoccurrence and resistance after treatment, it has earned itself the reputation of being an undruggable molecule. Several small molecule inhibitors are being developed to target this seemingly undruggable mutant. Recently, Sotorasib (AMG 510) has been identified as a potential small molecule inhibitor of the KRAS G12C mutation. In a recent phase 1 trial monitoring the dosage and safety of the treatment, 129 patients diagnosed with various cancer types were used to detect the effectiveness of the drug. The study had a majority of 59 patients with Non-small-cell lung carcinoma (NSCLC). The drug was administered orally with monotherapy- meaning only the single drug and nothing else- once daily. In the subgroup with NSCLC, 32.2% of patients showed an objective response to the drug (percentage of patients whose disease showed a positive response to the drug), and 88.1% had disease control (percentage of patients whose disease regressed or remained stable) [4]. Further testing on PDAC may give us valuable insight on the effectiveness of this small molecule inhibitor. Another drug, Adagrasib (MRTX849), was recently recognized as a potential inhibitor of the $KRAS^{G12C}$, the most common mutation of KRAS. It is the first of all $KRAS^{G12C}$ mutation inhibitors to move to clinical trials. *In vitro*, tumor regression was observed in 17 of 26 (65%) patient-derived xenograft models (tissue taken from humans and placed into mice) that had $KRAS^{G12C}$ mutations at dose levels of 30 -100 mg/kg/day of Adagrasib. Adagrasib caused significant tumor reduction in lung and colon adenocarcinoma patients, indicating the need for further testing of this inhibitor in PDAC [5].

III. NF-κB Pathway

NF-κB is an especially important transcription factor that plays diverse roles in PDAC, including inflammation, angiogenesis, control of apoptosis, and proliferation through transcription of various cytokines, leading to cancer-related inflammation. Constitutive NF-κB activation is present in 90% of cancers, including PDAC [6]. Studies show that mutant KRAS and several cytokines activate NF-κB resulting in the expression of proinflammatory signals. The mechanism by which NF-κB is activated by KRAS is shown in Figure 1. First, KRAS activates AP-1. In response to the activation of AP-1, an upregulated transcription factor in mutated KRAS cells, Interleukin-1α (IL-1α), a cytokine, is overexpressed. IL-1α then activates the IKK2/B complex, which causes the consequent expression of NF-κB. This is the short term activation of NF-κB. In order to sustain this activation long term, NF-κB then continually results in the expression of p62 and IL-1α, which act in a feedforward loop in order to keep NF-κB activated long term. Thus, proinflammatory and

antiapoptotic responses are achieved in KRAS mutated and NF-κB activated cells through the auto-regulatory loop caused by IL-1α and p62 [7].

Figure 1: NF-κB activation in KRAS mutated tumor cells through p62 and IL-1α feedforward loop

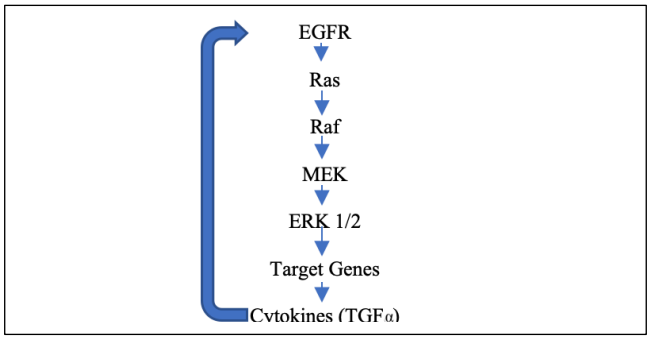


This pathway illustrates that inhibition of IL-1α overexpression can hinder PDAC development by suppressing NF-κB activation because NF-κB is a downstream effector of IL-1α. The mechanism by which IL-1α induces the NF-κB pathway is through the IL-1R receptor. The IL-1 acts as a link between the KRAS mutation and the NF-κB pathway needed for mutant KRAS induced PDAC, making it an important therapeutic target. A recent study showed that Anakinra, an FDA approved treatment, inhibits the IL-1 receptor and can decrease tumor progression by inhibiting IL-1α induced NF-κB activity. The study used various human PDAC lines as well as testing *in vivo* on orthotopic nude mouse models, genetically engineered mice implanted with tumors, (n=20, 5 per group) to demonstrate the increased effectiveness of Anakinra in decreasing tumor growth along with the potential benefits of using it alongside chemotherapy (Gemcitabine, the most common and effective chemotherapeutic treatment currently) [8]. TPCA-1 (GW683965) is also found to be a potential treatment due to its direct inhibition of the IKK2/B complex upstream of NF-κB. The study, using tumor xenograft models, also found that through the dual inhibition of NF-κB and another simultaneous tumorigenic pathway (STAT3), the inhibitor represses the transcription of IL-6. This can have profound effects on the autocrine mechanism in which the NF-κB acts [9]. BAY 11-7082 (BAY) is another small molecule inhibitor that acts similar to TPCA-1 by inhibiting the IKK complex upstream of NF-κB. It has profound effects on tumor development by acting as an anti-inflammatory protein, which is key to tumor regression. Moreover, BAY demonstrated broad-spectrum inhibition of other oncogenic cellular pathways including PI3K/Akt/IKK/NF-κB, ERK/JNK/AP-1, TBK1/IRF-3, and JAK-2/STAT-1 [10]. Further testing may reveal the extensive power BAY 11-7082 has in fighting PDAC.

IV. MAPK/ERK Pathway

The MAPK pathway plays a key role in cell differentiation and proliferation. Mutational Ras, activated by the binding of a signaling ligand on the Epidermal growth factor receptor (EGFR), causes the activation of downstream effectors such as Raf and eventually MAPK, also called MEK in mammals. MEK can also activate Extracellular signal-regulated kinase (ERK), which moves to the nucleus to activate a variety of transcription factors, ultimately leading to the transcription of various cytokines and cell survival. Furthermore, ERK activation can result in the upregulation of Transforming growth factor alpha (TGFα), and EGFR ligand, creating an autocrine feedback loop [11].

Figure 2: MAPK/ERK activation in KRAS Mutated tumor cells through Raf



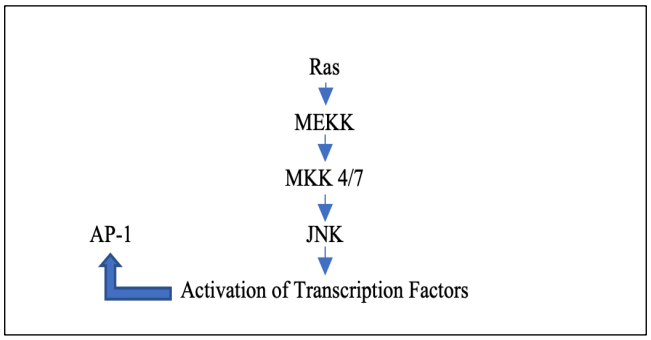
Potential small molecular inhibitors of this pathway are still in pre-clinical evaluations, but some drugs show promise. The Raf inhibitor, LY3009120, prevents the downstream phosphorylation and activation of MEK/ERK in Ras mutated melanoma. In nude mice with Patient-derived xenografts (PDX) tumors, a dosage of 15 to 30mg/kg of LY300912 showed almost complete tumor regression with no toxicity observed [12]. Downstream of Raf, CI-1040 (PD 184352) is the first MEK inhibitor shown to inhibit tumor growth *in vivo*. The drug was administered once daily with a dosage of 100 mg/day up to 1600 mg/day in a Phase 1 trial with a sample size of 66 patients. 1 patient showed partial response for 12 months, and 19 patients showed a stable disease lasting a median of 5.5 months [13]. Unfortunately, the Phase II trial showed no significant response worth noting [14]. A variation of CI-1040 (PD 184352) known as PD 0325901 was developed after the failure of the first drug. Compared to CI-1040, PD 0325901 had a 70-fold increase in potency in pre-clinical evaluations. Patients received doses of up to 20 mg and in a sample size of 27 patients, 2 patients were reported to have partial response and 8 patients had stable disease [15]. A third MEK inhibitor, ARRY-142886 (AZD6244) shows promise as it shows potent activity in human tumor xenograft models [16]. Overall, MEK Inhibition shows promise as stable disease is observed in patients with various types of cancer and it is warranted for further research. In some cases, MEK inhibition can be toxic, and it might be important to consider the ablation (removal) of the upstream receptor, EGFR, instead. Studies show that mutated KRAS upregulates EGFR expression, and the inhibition and ablation of EGFR significantly decreases tumorigenesis *in vivo* because EGFR is necessary for MEK/ERK activation [17]. That being said, a recent study showed that patients with KRAS mutations (181 patients) showed less improvement with targeted EGFR treatment than patients without (86 patients), pointing to the need for more understanding of this approach [18]. In contrast, targeted deletion of both EGFR and Raf prevented PDAC development in KRAS driven tumor models as well as

human PDX models for 2 years with no precursors to tumor progression, while also not affecting downstream MEK/ERK activation, eliminating the need to worry about treatment-related toxicity [19].

V. JNK Pathway

The c-Jun NH_2-terminal kinase (JNK) Pathway has a dualistic role in cancer development because it has both pro and anti-tumor functions. Ras acts as a switch converting JNK from having an anti-tumor role to a pro-tumor role. JNK is usually activated by stress cytokines such as IL-1α and tumor necrosis factor α (TNF-α) [20]. Ras activates downstream effectors, mitogen-activated extracellular signal-regulated kinase (MEK), Mitogen-activated protein kinase (MAPK) kinase 4 or 7, and eventually JNK. JNK causes the transcription of Activator Protein-1 (AP-1) commonly implicated in cell proliferation and differentiation [21]. This pathway is clearly shown in Figure 3. Dysregulation of the JNK pathway may increase cell proliferation [22]. Several loss of function studies have shown that JNK activation is necessary for Ras-induced transformation of tumor cells [23].

Figure 3: JNK activation pathway in KRAS Mutated tumor cells

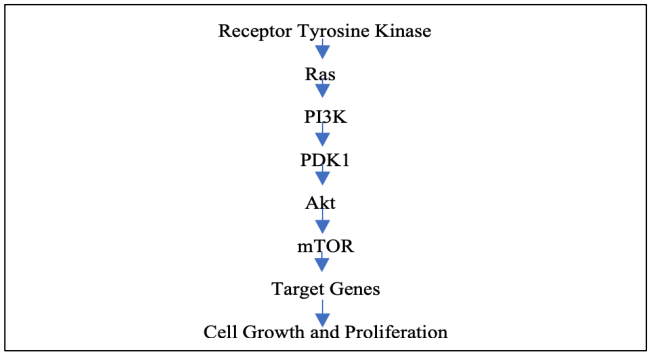


Because JNK is implicated in many different cancers, therapeutically targeting JNK is in the best interest for PDAC patients [24]. SP600125, a selective inhibitor, is shown to significantly inhibit JNK and prevent the activation of many downstream cytokines. SP600125 was administered at dosages up to 15 mg/kg in female CD-1 mice. SP600125 showed significant results with high specificity to the JNK pathway [25]. An IC50 of a drug helps indicate the dosage of the drug necessary to reduce the response by half. The IC50 of SP600125 for JNK2 is equal to 40 nM and for JNK3, it is 90 nM [26]. A recent study investigated the efficiency of another small molecule inhibitor, Bentamapimod (AS602801), of the JNK pathway. Bentamapimod has been shown to be effective in the treatment of inflammatory endometriosis. The study explored its effects on human cancer cells both *in vitro* and *in vivo*. In cancer stem cells derived from human pancreatic cancer, Bentamapimod demonstrated cytotoxicity [27]. AS602801 has an IC50 for JNK1 = 80 nM, for JNK2 = 90 nM, and for JNK3 = 230 nM.

VI. PI3K Pathway

The PI3K pathway plays a role in cell metabolism, growth, and proliferation. PI3K is bound to Receptor Tyrosine Kinases (RTKs) in the cell membrane and activation of the receptor by an extracellular signal activates PI3K to convert Phosphatidylinositol (3,4)-bisphosphate (PIP_2) lipids into Phosphatidylinositol (3,4,5)-trisphosphate (PIP_3). Mutated Ras facilitates the binding of phosphoinositide-dependent kinase-1(PDK1) to PIP_3 at the cell membrane causing the downstream activation of Protein kinase B (Akt) and Mammalian target of rapamycin (mTOR) as shown in Figure 4 [28]. The PI3K/AKT signaling pathway is one of the most deregulated pathways in cancer and is commonly found in KRAS driven-pancreatic cancer both in human PDAC and mouse models [29][30].

Figure 4: PI3K activation in KRAS Mutated tumor cells



PI3K therapeutic treatments have been unsuccessful thus far as the specificity of the molecules and the toxicity at which they have worked has limited their potential [31]. However, several inhibitors are in Phase III of clinical trials. Gedatolisib (PF04691502), a dual PI3K/mTOR, has shown potent activity and decreased tumor density in head and neck squamous cell carcinomas by inhibiting Akt proliferation. The comparison between control tumor cell lines and those treated with Gedatolisib using an XTT assay after 48 hours showed statistical significance with P value ≤ 0.01 [32]. BKM120 (Buparlisib) is an orally administered reversible PI3K inhibitor. Both *in vitro* and *in vivo*, Buparlisib shows significant antiproliferative activity [33]. A maximum tolerated dose (MTD) of 100 mg/day showed efficiency as well as safety in a study with sample size of 64 patients [34]. This study proved an earlier study testing Buparlisib on 15 Japanese patients with advanced solid tumors and another study with 35 Western patients that also settled on 100 mg/day as the most efficient dose [35][36].

Conclusion

From an improved understanding of the dysregulated cell-signaling pathways mentioned (NF-κB, MAPK/ERK, JNK, and PI3K), many targeted therapeutic treatments have emerged in the past decade. The high mutation rate and inherent drug resistance of PDAC serves as a barrier to effective treatment. This paper elucidates some of the most novel treatments that hold potential to target parts of these mutated pathways as a framework to combat tumor progression, but much remains to be learned on how to effectively utilize these treatments to target KRAS mutated pancreatic tumors in patients. Note that most pathways act

synergistically through cross talk, so targeting many parts of different pathways may offer better treatment plan clinically. For example, NF-κB and JNK share common upstream activators and may act synergistically to regulate cancer cell survival. Looking ahead, it may be valuable to research the effects of a combination of the small molecule inhibitors given for more effective treatment. The small molecule inhibitors discussed can also be combined with dosages of the current effective chemotherapeutic drugs for PDAC, Gemcitabine and 5-fluorouracil (5-FU). Gemcitabine has already shown promise when combined with Anakinra as mentioned above. Applying targeted therapy to the tumor microenvironment is another approach that can be researched. Often the tumor microenvironment with its dense stroma and inflammatory cells makes drug delivery ineffective, so combining small molecule inhibitors with immunotherapy might be a more effective course of treatment. As of now, there is only one FDA approved immunotherapeutic drug for PDAC, and it is Pembrolizumab (Keytruda). Utilizing it along with targeted therapy in patients with PDAC is worthy of consideration. Combination therapy holds a lot of promise in the treatment of PDAC, but it is also important to consider the toxicity that occurs when multiple drugs are administered. Further studies using small molecule inhibitors with other treatments must be conducted before deeming this as an effective course of treatment.

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