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Is There Potential to Target FOXM1 for “Undruggable” Lung Cancers?

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Abstract

Published studies with transgenic mice convincingly showed that FOXM1 transcription factor is an important component of the KRAS/ERK signaling pathway in respiratory epithelial cells. FOXM1 is required for oncogenic KRAS signaling in mouse lung cancer models and therefore, clear potential exists to target FOXM1 in human non-small cell lung cancers (NSCLC) driven by activated KRAS mutations. To date, several approaches to inhibit FOXM1 in cancer cells have been explored. These include siRNA/shRNA-mediated inhibition of Foxm1 mRNA, sequestration of FOXM1 protein in nucleoli using ARF peptide, inhibition of FOXM1 binding to its target promoter DNAs by the FDI-6 small molecule compound and inhibition of proteasomes by thiazole antibiotics. Additional studies are needed to determine if inhibition of FOXM1 is beneficial for treatment of KRAS mutant NSCLCs in human patients and to develop effective delivery systems for FOXM1 inhibitors. If successful, additional strategies can be explored to screen for novel FOXM1 inhibitors, such as targeting FOXM1 nuclear localization, nuclear export or protein-protein interactions with activating kinases and co-activator proteins. Altogether, inhibition of FOXM1, either alone or in combination with other anti-cancer drugs, could be beneficial for treatment of KRAS mutant NSCLCs that are resistant to conventional chemotherapy.

Keywords

FOXM1; KRAS; non-small cell lung cancer; NSCLC; transgenic mice

Published studies demonstrated that FOXM1 protein (also known as HFH-11B, Trident, Win or MPP2) is upregulated in a variety of human cancers, including non-small cell lung cancer (NSCLC), hepatocellular carcinoma, glioblastoma, as well as pancreatic, breast and prostate adenocarcinomas (reviewed in [1]). FOXM1 is a transcription factor from the Forkhead Box (FOX) family, which includes over 50 related mammalian proteins. In vitro and in vivo studies convincingly showed that FOXM1 stimulates proliferation of tumor cells by activating transcription of multiple cell cycle regulatory genes, including Cdc25B, cyclins

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B1 and A2, Polo-like and Aurora B kinases [1,2]. There is a positive correlation between increased FOXM1 and poor prognosis in cancer patients [3]. Numerous studies with mouse cancer models support an oncogenic role of FOXM1 in cancer cell proliferation, invasion and tumor growth. For example, aberrant expression of FOXM1 in *Rosa26-FoxM1* transgenic mice accelerated proliferation of tumor cells and increased the number and size of lung tumors after treating mice with 3-methylcholanthrene (MCA)/ butylated hydroxytoluene (BHT), a known model of lung tumor initiation/ promotion [4]. Likewise, genetic deletion of the *Foxm1* gene from all cell types (*Mx-Cre Foxm1^{-/-}* mice) or respiratory epithelial cells (*SPC-rtTA/TetO-Cre Foxm1^{-/-}* mice) inhibited lung tumorigenesis induced by either MCA/ BHT or urethane [5,6], both of which cause a high frequency of activating mutations in the *Kras* oncogene. Supporting an oncogenic role of FOXM1 in lung cancers, genetic deletion of *Foxm1* from respiratory epithelial cells completely abrogated the initiation of lung tumorigenesis by activated *Kras^{G12D}* transgene [7]. These results indicate that FOXM1 functions downstream of oncogenic KRAS to induce formation of lung tumors. Consistent with these studies, several KRAS-regulated kinases, including Cdk1, Cdk2, Cdk4, Cdk6 and ERK, were capable of phosphorylating and activating FOXM1 protein in cultured tumor cells (reviewed in [1]). Interestingly, deletion of the *Foxm1* gene prevented the aberrant effects of activated *Kras^{G12D}* during lung development [8]. All these published studies suggest that FOXM1 is required for oncogenic KRAS/ERK signaling in both normal and neoplastic lung epithelial cells, raising a hypothesis that pharmacological targeting of FOXM1 could be useful for therapy in lung cancer patients with activating KRAS mutations.

Mutations in the *KRAS* gene are frequently found in human lung, colon and pancreatic adenocarcinomas [9]. Up to 30% of patients with lung adenocarcinomas are positive for KRAS mutations that usually affect exon 2 and 3, causing accumulation of the RAS protein in the active GTP-bound state. This results in activation of the RAS downstream signaling cascade, including phosphorylation of the mitogen-activated protein kinases MAPKs and activation of the PI3K/Akt/mTOR and the RAL pathways, ultimately stimulating cellular proliferation and inhibiting apoptosis in tumor cells. KRAS mutations are associated with tobacco use and KRAS mutant NSCLCs have poor prognosis [10]. Current treatment of KRAS mutant NSCLCs is very challenging due to resistance to common anti-cancer drugs. KRAS mutant NSCLCs are routinely treated with platinum-pemetrexed doublet or carboplatin/ paclitaxel/ bevacizumab as the first-line therapy, followed by pemetrexed maintenance therapy [10]. Unfortunately, targeted therapy against mutant RAS proteins is not available and targeting KRAS downstream targets, such as RAF, MEK and ERK, thus far not shown significant clinical benefit in KRAS mutant NSCLCs. Based on the critical importance of FOXM1 for KRAS signaling in mouse lung cancer models, inhibition of FOXM1, either alone or in combination with other anti-cancer drugs, could be beneficial for treatment of NSCLCs with activating mutations in the *KRAS* oncogene.

FOXM1 is a nuclear protein without known enzymatic activity, and therefore, it is considered an “undruggable” target. However, several recent studies have proven this assumption to be wrong. Discovery of protein-protein interactions between FOXM1 and the P19^{ARF} tumor suppressor, led to development of the ARF peptide, which specifically binds to the FOXM1 protein and sequesters it in nucleoli, thereby inhibiting FOXM1

transcriptional activity [11]. To ensure membrane-penetrating properties of the ARF peptide, it was fused to nine N-terminal D-Arg residues that enhance cellular uptake [11]. Administration of ARF peptide to mice bearing hepatocellular carcinomas (HCC) inhibited HCC progression through specific binding and inactivation of the FOXM1 protein [12]. Another recent study demonstrated that the ARF peptide was capable of inhibiting the FOXM1 protein in a mouse model of asthma [13]. Although these studies clearly demonstrate the efficacy of the ARF peptide in vivo, long-term administration of the peptide can lead to unwanted immunological responses to the peptide structure, potentially decreasing its effectiveness in cancer patients. Another potential problem is delivery of the ARF peptide to the lung tumor region. The ARF peptide was effectively delivered to the liver through intra-peritoneal injection [12] and was capable of penetrating airway epithelium and a subset of alveolar macrophages after intra-tracheal administration [13]. However, delivery of ARF peptide to peripherally located lung tumors will be technically challenging, and probably require the development of specialized delivery systems, such as nanoparticles. Interestingly, the ARF peptide effectively induced apoptosis of HCC cells in vivo [12], whereas apoptosis was not observed after Cre-mediated deletion of the *Foxm1* gene from hepatocytes [1]. These findings indicate that the ARF peptide has an off-target effect, which is distinct from inactivation of FOXM1. Additional studies are needed to determine whether pro- or anti-apoptotic pathways are targeted by the ARF peptide in tumor cells. All these potential limitations make the use of ARF peptide impractical, at least, for the purposes of inhibiting FOXM1 in KRAS mutant lung tumors.

FOXM1-specific siRNA and shRNA were recently found to be effective in inhibiting FOXM1 protein in a mouse xenograft breast cancer model [14] but this approach was not tested in KRAS mutant lung tumors. Delivery system(s) to effectively target lung tumors with siRNA/shRNAs have not yet been developed. The ideal system will likely consist of encapsulated nanoparticles that can effectively accumulate in lung tumors without targeting normal lung tissue or other organs. Since FOXM1 is highly expressed in small intestine, colon and thymus and critically important for function of these organs [1], accumulation of FOXM1 inhibitors in these tissues will be undesirable and could potentially lead to aberrant gastrointestinal and immunological reactions. In addition, the chronic use of FOXM1 inhibitors could interfere with normal repair process in response to microbial agents or environmental stimuli.

Several other FOXM1 inhibitors have been recently described. Thiazole antibiotics Siomycin A and thiostrepton inhibited FOXM1 in cultured tumor cells and in mouse tumor models (reviewed in [14]). Both of these agents were found to be proteasome inhibitors and, therefore, stabilized the expression of multiple cellular proteins. Although physical interaction between thiostrepton and the FOXM1 protein has been reported, there is no direct evidence that thiostrepton-FOXM1 interactions play a role in reducing FOXM1 transcriptional activity. Due to proteasome inhibiting properties of thiazole antibiotics, it is likely that these agents will affect multiple signaling pathways in addition to inhibition of FOXM1. In fact, inactivation of the NF- κ B signaling pathway has been linked to the use of proteasome inhibitors [14]. Most recently, a novel small molecule compound, FDI-6, has been described as a FOXM1 inhibitor [15]. FDI-6 was shown to directly bind to FOXM1 protein and block its binding to DNA. Interestingly, FDI-6 specifically inhibited FOXM1

target genes and did not influence gene expression regulated by other forkhead transcription factors [15]. However, since FOXM1 and other forkhead proteins share DNA-binding sites, it is difficult to imagine that inhibition of *forkhead* domain/DNA binding could produce a very specific FOXM1 inhibitor. Therefore, specificity of FDI-6 to FOXM1 should be rigorously investigated and if confirmed, the FDI-6 compound can potentially be used to inhibit FOXM1 in KRAS mutant lung tumors. Additional studies are needed to assess toxicity and bioavailability of this small molecule compound in animal cancer models.

In summary, FOXM1 transcription factor is required for oncogenic KRAS signaling in cultured tumor cells and mouse lung cancer models. Based on these studies, there is a clear potential for targeting FOXM1 in human NSCLC cancers with activated KRAS signaling. FOXM1 activity in tumor cells can be inhibited by targeting the FOXM1 protein to nucleoli (the ARF peptide), preventing interactions of FOXM1 with DNA (the FDI-6 small molecule compound) and inhibiting proteasomes in tumor cells (Siomycin A and thiostrepton). Additional strategies to screen for novel FOXM1 inhibitors can be explored, such as targeting FOXM1 nuclear localization, nuclear export or protein-protein interactions with activating kinases and co-activator proteins. Inhibition of FOXM1, either alone or in combination with other anti-cancer drugs, could be beneficial for treatment of KRAS mutant NSCLCs that are resistant to conventional chemotherapy.

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Abbreviations

NSCLC	non-small cell lung cancer
Cre	Cre recombinase
FOXM1	Forkhead Box transcription factor M1

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