Selective requirement for Mediator MED23 in Ras-active lung cancer

Xu Yang, Meng Zhao, Min Xia, Yuting Liu, Jun Yan, Hongbin Ji, and Gang Wang

State Key Laboratory of Cell Biology, Institute of Biochemistry and Cell Biology, and Key Laboratory of Computational Biology, Chinese Academy of Sciences-Max Planck Institute Partner Institute for Computational Biology, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China

AUTHOR SUMMARY

Lung cancer is the leading cause of cancer-related mortality worldwide and arises from the accumulation of multiple oncogenic lesions. A point mutation in the Ras gene family member, the K-Ras gene, and constitutive activation of the Ras–MAPK signaling pathway are involved in certain lung cancers (1). Efforts to develop Ras-directed therapies are challenged by the difficulty in selectively targeting the activated Ras GTPase (2). Here, we screened a panel of lung cancer cells with and without Ras mutations and demonstrated that MED23, a component of the Mediator complex, and its interacting transcription factor, ELK1, are critical for Ras-driven oncogenesis and may provide potential targets for “undruggable” Ras-driven lung cancer.

Mediator is an evolutionarily conserved cofactor complex of the RNA polymerase II machinery that functions as an integrative hub to coordinate signaling pathways and gene activities that direct diverse biological processes (3). The Mediator MED23 subunit was considered a downstream nuclear factor for the Ras–MAPK signaling pathway (4). In this study, we examined the role of MED23 in lung cancer. Using viral-mediating shRNA to inhibit Med23 expression in a large panel of human lung cancer cell lines with or without a Ras mutation, we found that depletion of Med23 selectively inhibited the proliferation and tumorigenicity of cancer cells with mutated Ras but not with wild-type Ras. Introducing the mutated Ras into the cell lines with wild-type Ras renders them dependent on MED23 for growth. Thus, our finding indicates that MED23 controls the proliferation of lung cancer cells in a Ras-dependent manner.

To establish further the direct relationship between MED23 and Ras during oncogenesis, we used oncogenic transformation assays to model the MED23 dependence in fibroblast transformation by different oncogenes. We found that Med23 deficiency in fibroblasts selectively inhibited oncogenic transformation by Ras but not by c-Myc. Moreover, the expression level of Med23 was highly up-regulated during the transformation by Ras but less so by other oncogenes, including c-Myc and T-antigen, further confirming the specific codependency of active Ras and MED23 in Ras-driven oncogenesis.

Genetic and biochemical interaction studies demonstrated that MED23 is targeted specifically by the ternary complex factor ELK1 for channeling the Ras/MAPK signaling to the nucleus (5). We asked whether Mediator MED23 controls Ras-driven oncogenesis and downstream gene expression through its binding partner, ELK1, and found that ELK1 was also selectively required for the cancer cell proliferation and fibroblast transformation in a Ras-dependent manner. Thus, ELK1 seems to reiterate the phenotype specified by Med23 in regulating tumorigenesis. Further, to gain an understanding of how MED23 and ELK1 control gene expression in lung cancer cells harboring activated Ras, gene-profiling experiments were performed and revealed that MED23 and ELK1 exerted similar effects on global gene regulation and coreregulated a set of genes involved the cell cycle. These findings suggest that interaction between MED23 and ELK1 is important for transduction of the oncogenic Ras signaling and may account for the Ras-driven oncogenesis.

To understand further the function of MED23 in Ras-driven tumorigenesis, we determined the expression of MED23 in a series of lung cancer cell lines and in a large panel of clinical lung cancer samples. Consistent with findings for cell lines transformed by Ras, MED23 was found to be overexpressed in lung cancer cell lines and in lung cancer samples, and this increased level of expression corresponded to the active Ras activities indicated by ERK- and ELK1-phosphorylation. Therefore, the elevation in MED23 expression was not only required for but also resulted from Ras hyperactivity during lung carcinogenesis, suggesting their mutual dependence. Remarkably, we found that the expression level of Med23 correlated specifically with clinical outcomes of patients with a positive Ras signature (i.e., a gene-expression profile associated with mutated Ras). Specifically, lower Med23 expression levels predict better survival in lung cancer patients with a positive Ras signature. This observation was recapitulated further in a xenograft mouse model with an inducible Med23 knockdown strategy. Taken together, our results suggest that MED23 may serve as a diagnostic marker and a therapeutic target for individualized therapy for lung cancers harboring activated Ras.

Author contributions: X.Y. and G.W. designed research; X.Y., M.Z., and M.X. performed and revealed that MED23 and ELK1 exerted similar effects on global gene regulation and coreregulated a set of genes involved the cell cycle. These findings suggest that interaction between MED23 and ELK1 is important for transduction of the oncogenic Ras signaling and may account for the Ras-driven oncogenesis.

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Our study revealed that MED23 senses and relays the hyperactive Ras signaling to the downstream target genes through interacting with the phosphorylated ELK1, thus controlling the cancer cell proliferation and tumorigenicity. The selective requirement for MED23 in lung cancer may depend on the strength of the Ras/MAPK signaling as indicated by ERK- and ELK1-phosphorylation levels. Moreover, these findings suggest that the malignant phenotype of cancer cells harboring K-Ras mutations is dependent not only on the oncogenic K-Ras but also on the entire Ras/MAPK pathway, including the downstream cofactor MED23. Thus, the model of “oncogenic pathway addiction” may better describe the up-regulation of the entire Ras/MAPK pathway by the oncogenic Ras mutation, including ELK1/MED23 as key modulators and effectors within the nucleus (Fig. P1). In other words, Ras signaling is dependent on ELK1 and MED23 for its addiction. Because multiple often-mutated oncogenes in the Ras/MAPK pathway, such as EGFR, Ras, and Raf, share a common signal transduction cascade, patients with mutations in any of these oncogenes might benefit from targeting MED23. Indeed, we have observed that the depletion of Med23 can inhibit the growth of cancer cells with EGFR or B-raf mutations also. Knockdown of Med23 or Elk1 attenuates the transduction of the oncogenic signaling to the nucleus; therefore, targeting MED23, ELK1, or their interaction interface by specific inhibitors may represent multiple opportunities to treat lung cancer or other types of cancer harboring hyperactive Ras signaling for which there is no effective therapy.