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A Mediator Lost in the War on Cancer

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An unexpected role for a Mediator subunit, MED12, in resistance to multiple anticancer agents is revealed by Huang et al. Loss of MED12 confers drug resistance by activating transforming growth factor β (TGF-β) signaling. Inhibition of the TGF-β pathway resensitizes cells to therapeutic drugs, suggesting a new combinatorial cancer treatment.

Carcinogenesis is frequently associated with aberrant kinase activities in transformed cells. Potent and specific kinase inhibitors represent an important component of targeted cancer therapy, which has become part of many cancer treatment regimens because of its precision in killing cancer cells with relatively few side effects as compared to traditional chemotherapies. In the anticancer war, however, precision may also mean narrowness, which is an intrinsic drawback of targeted cancer therapy because it often allows cancer cells to regroup, that is, develop drug resistance, one of the primary reasons for treatment failure. In this issue, Bernards and colleagues report the results of a screen with short hairpin RNA (shRNA)-mediated knockdown that identifies a common determinant of drug resistance in several cancer cell lines (Huang et al., 2012) (Figure 1).

Our knowledge on cancer drug resistance is far from complete due to the complexity of the disease and variation among patients. In some cases, drug resistance is inherent to cancer cells but can also be acquired under selective pressure via a number of distinct mechanisms (Gottesman, 2002). Understanding these mechanisms can yield great clinical benefits for predicting patient responses and devising alternative treatment strategies (Bock and Lengauer, 2012).

Large-scale screens in cancer cell lines with expression clones, shRNAs, or small compounds are widely used to identify factors that confer or prevent resistance to a particular anticancer treatment. In the current work, Huang et al. started with a lung cancer line harboring a translocation between *EML4* and the kinase *ALK*, which are sensitive to ALK inhibitors. They screened 24,000 shRNAs targeting 8,000 human genes in the hope of discovering gene products that would enable cancer cell growth in the presence of the drugs. One particular hit met their stringent criteria, and strikingly, knockdown of the gene in different types of cancer cells leads to broad drug resistance. These cells continue to grow in the presence of inhibitors against receptor tyrosine kinases (RTKs), BRAF, and MEK and are still capable of maintaining relatively high activity of ERK.

The gene target identified is an unexpected one, *MED12*, which encodes a subunit of the Mediator complex that is essential for gene transcription in all eukaryotic cells. Consisting of at least 26 subunits, the Mediator complex is a dynamic and sophisticated regulatory unit that has been extensively studied for its role in chromatin remodeling, transcription factor recognition, RNA polymerase II (Pol II) recruitment and stabilization, and transcription initiation/elongation (Taatjes, 2010). Several Mediator subunits, including cyclin-dependent kinase 8 (CDK8), CYCLIN C, MED12, and MED13, form the so-called CDK8 submodule that reversibly interacts with Mediator. The CDK8 complex can both positively and negatively regulate gene transcription and has been implicated in carcinogenesis (Taatjes, 2010).

How does MED12, a subunit of the nuclear CDK8-Mediator complex, regulate cytoplasmic signal transduction from RTKs to ERK and control responses to anticancer drugs? To answer this question, Huang et al. performed a second round of shRNA screen specifically looking for kinases that are necessary for the acquired drug resistance in MED12 knockdown cells. Again, they identify one target that fulfills all the criteria of the screen, and that gene encodes the type II receptor of transforming growth factor β (TGF-βR2). Depletion of TGF-βR2 restores drug sensitivity of MED12-deficient cancer cells.

In the canonical TGF-β pathway, TGF-βR2 activates TGF-βR1, another receptor serine/threonine kinase, which in turn phosphorylates transcription factors SMAD2 and SMAD3. The Smads then translocate to the nucleus and regulate the expression of TGF-β target genes. A non-SMAD pathway also exists that transduces signals from TGF-βRs to MAPKs such as ERK (Massagué, 2012). In fact, Huang et al. find that MED12 knockdown in the cancer cells causes an upregulation of TGF-βR2 protein that is sufficient to trigger downstream signaling, both SMAD dependent and independent. ERK is therefore activated by this alternative mechanism, which to a large extent explains the observed drug resistance to RTK inhibitors.

Yet how does MED12 negatively control TGF-βR2 protein levels? The authors make another surprising discovery that MED12 is also present and functions in the cytoplasm where TGF-βR2 resides. This property appears to be unique to MED12 as other components of the CDK8 submodule are not found outside the nucleus, nor are they involved in regulating drug resistance. Imaging and biochemical data suggest that MED12 physically and preferentially binds the immature form of TGF-βR2 during its secretion and somehow prevents its glycosylation (which is required for TGF-βR2 function) and/or delivery to the cell surface. More details of this regulation

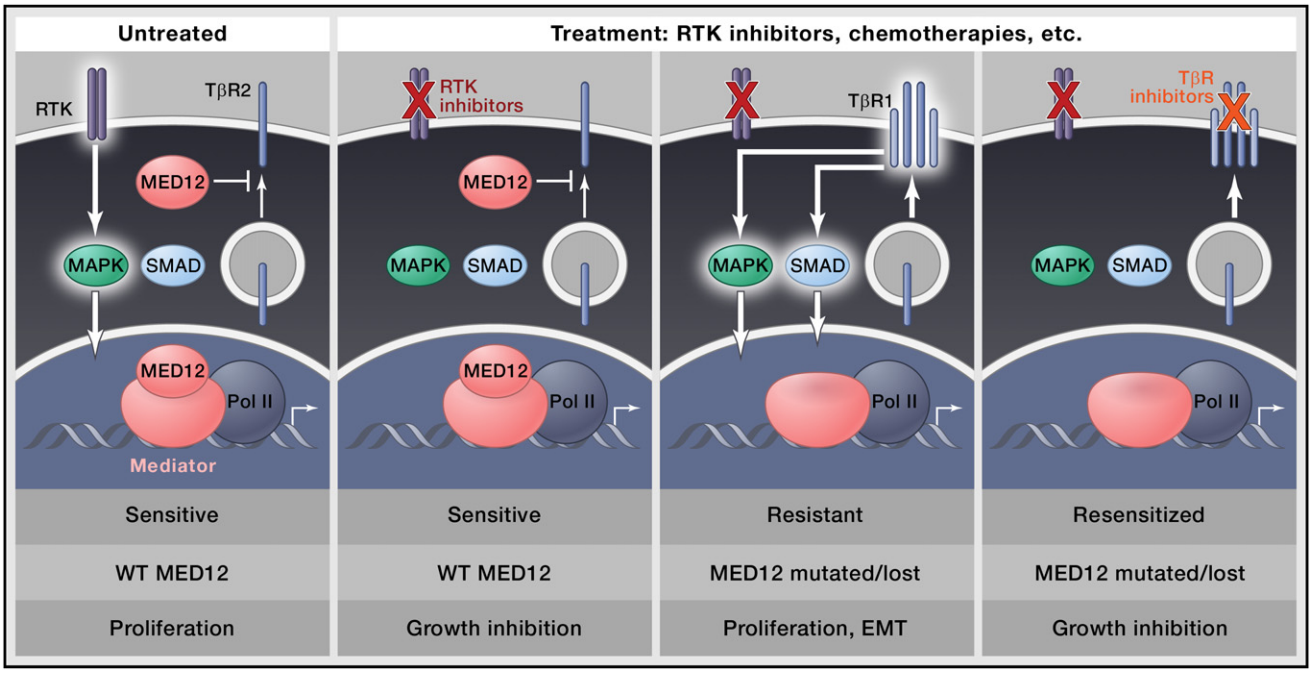

Figure 1. MED12 and Cancer Drug Resistance

Cancer cells harboring hyperactive RTKs are normally sensitive to RTK inhibitors. shRNA-mediated knockdown of the Mediator subunit MED12 causes drug resistance by increasing the level of TGF-βR2 (the type II receptor of transforming growth factor β), which activates the ERK and SMAD pathways. This leads to cell proliferation and features of EMT. Inhibition of TGF-βRs inactivates ERK and SMADs, resensitizing MED12-deficient cells to anticancer agents.

remain to be delineated, especially where the interaction occurs in the secretory pathway and how TGF-βR2 maturation is affected by MED12. Nevertheless, the findings clearly represent an intriguing mechanism for modulating TGF-β signaling and make one wonder whether MED12 is also capable of controlling other receptor proteins in a similar manner.

Another significant observation from this study is that drug-resistant MED12 knockdown cancer cells exhibit a unique gene-expression signature that is both prognostic and predictive. First, this signature shows features of epithelial-to-mesenchymal transition (EMT), a process strongly induced by TGF-β and often associated with poor prognosis. Indeed, colorectal cancer patients bearing the MED12 knockdown signature have worse clinical outcomes than those with a wild-type MED signature. Whether EMT has a causal role in drug resistance is an interesting question that remains to be addressed. Second, therapeutic drugs such as MEK inhibitors, EGFR inhibitor, and 5-Fluorouracil are more likely to fail if the cancer cells show a MED12 knockdown pattern of gene expression. Given that double depletion/inhibition of MED12 and TGF-βR2 causes synthetic lethality of cancer cells in the screen, a personalized combinatory therapy (Kummar et al., 2010) with both TGF-βR inhibitors and RTK inhibitors is worth exploring for treating patients with markers of MED12 knockdown.

In MED12-deficient cancer cells, neither the TGF-βR2 inhibitor nor RTK inhibitors alone block growth, but they do so synergistically. In addition, the authors note that TGF-β treatment alone impedes cancer cell proliferation, whereas it provides a selective advantage only when the cells are challenged with anticancer drugs. These observations reiterate two important features of TGF-β signaling: (1) it constantly cross-talks with other pathways (Guo and Wang, 2009), and (2) it plays a dual role in cancer formation and progression (Massagué, 2012). It will be interesting to further dissect the individual and combined contributions of the SMAD and non-SMAD pathways to drug resistance and EMT in a broader setting and to identify the switch between the anti- and protumorigenic functions of TGF-β.

There are several other important questions regarding this new drug-resistance mechanism. First, MED12 is mostly point-mutated rather than deleted in human cancers (Mäkinen et al., 2011), and mutant MED12 is also implicated in noncancer genetic disorders. How is MED12 function altered by those mutations, and are those mutants also involved in cancer drug resistance? Second, CDK8 phosphorylates the linker region of SMAD2/3, which couples SMAD activation and degradation (Alarcón et al., 2009). It is unclear whether loss of MED12 would influence the nuclear function of SMAD proteins in a CDK8-dependent manner, which would in turn regulate cell proliferation, EMT, or responses to drugs. Third, can TGF-βR inhibitors be used to target cancer stem cells, which are notorious for their resistance to anticancer therapies (Dean et al., 2005)? Lastly, one obvious caveat of studies with cancer cell lines is that they cannot reflect the importance of the stroma and microenvironments within a tumor, which are also critical determinants of drug responses. Careful validation with in vivo models will be needed to convert these

exciting findings into effective weapons in the battle against cancer.

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Designing an Enhancer Landscape

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In this issue and in a recent issue of *Cell*, Vahedi et al. and Samstein et al. provide new insights into the strategies used to establish an enhancer landscape during development of cell lineages. They report that enhancer landscapes characterizing T cell lineages are pre-established and strongly influenced by environmental stimuli.

Transcription in eukaryotes is regulated by sequence-specific DNA-binding proteins associated with a gene's promoter, which encompasses the transcription start site, and also by one or more distant control regions, including enhancers. Enhancers typically bind several DNA-binding proteins and coregulatory proteins that modulate chromatin structure and directly communicate with the transcription machinery positioned at the promoter. Until recently, our knowledge was based on studies of only a small number of model enhancers because enhancers were difficult to identify at a genome-wide scale. During the past few years, postgenomic technologies have revealed characteristic features of poised and active enhancers that have facilitated enhancer discovery. By taking advantage of this newfound capability, Vahedi et al. (2012) and Samstein et al. (2012) in this issue and in a recent issue of *Cell* have expanded our knowledge of the diverse strategies used to activate enhancers during the development of mammalian cell lineages.

Vahedi et al. (2012) focused on active enhancers in two subtypes of mature helper T cells—Th1 and Th2 cells—which, in a simplistic view, promote immune responses to intracellular and extracellular microbial pathogens, respectively. These two cell types develop from the same naive Th cell precursor upon T cell receptor (TCR) engagement in the presence of different cytokine signals. Th1 development is catalyzed by IL-12 and IFN-γ, which activate the STAT4 and STAT1 transcription factors, respectively. Among the many genes activated by these STAT proteins in the naive Th cell is *Tbx21*, which encodes the T-bet transcription factor that is considered to be a master regulator of Th1 development. In contrast, Th2 development is catalyzed by IL-4, which activates the STAT6 transcription factor that cooperates with the Th2-specifying factor, GATA3.

To identify enhancers that are active in mature Th1 and Th2 cells, Vahedi et al. (2012) performed chromatin immunoprecipitation sequencing (ChIP-seq) analysis for the transcriptional coactivator and histone acetyltransferase, p300. The significance of p300 association is thought to be distinct from that of another prominent enhancer mark, monomethylation of histone H3 at lysine 4 (H3K4me1). H3K4me1 is thought to mark both active enhancers and inactive enhancers that are poised for activation, whereas p300 is more closely associated with active enhancers (Heintzman et al., 2007; Visel et al., 2009; Ghisletti et al., 2010).

The first surprise to emerge from this analysis was that a high percentage of p300-marked regions (excluding promoter regions) differed between the closely related Th1 and Th2 populations; 45% and 35% of p300 peaks were unique to Th1 or Th2 cells, respectively. Remarkably, extending the analysis to macrophages and embryonic stem cells