How to Drug "Undruggable" Cancers: Functional Genomics Points the Way

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The world of cancer research is abuzz with talk of the *MYC* family of transcription factor genes. The reason is simple. Because these genes regulate the expression of more than 15 percent of all human genes and carry out essential functions in proliferative tissues, their mis-regulation can drive a wide variety of cancers. For example, *MYC*oncogenes are associated with poor prognosis in breast, prostate and colon cancers. In addition, over-expression of one member of the gene family, *c-MYC*, is routinely found in a high proportion of ovarian, liver and lung cancers, and aberrant expression of *N-MYC*, another family member, contributes to pediatric neuroblastoma, the most common cancer afflicting infants. Clearly, a successful strategy to target *MYC*-driven cancers could have revolutionary implications for significant populations of cancer patients. Unfortunately, the solution is far from simple. *MYC* oncogene family members are considered "undruggable" for two reasons. First, *MYC* genes encode basic helix loop helix transcription factors (see figure), which do not possess druggable domains. Second, because *MYC*-encoded transcription factors carry out so many functions, their prolonged down-regulation would likely result in severe side effects for patients.

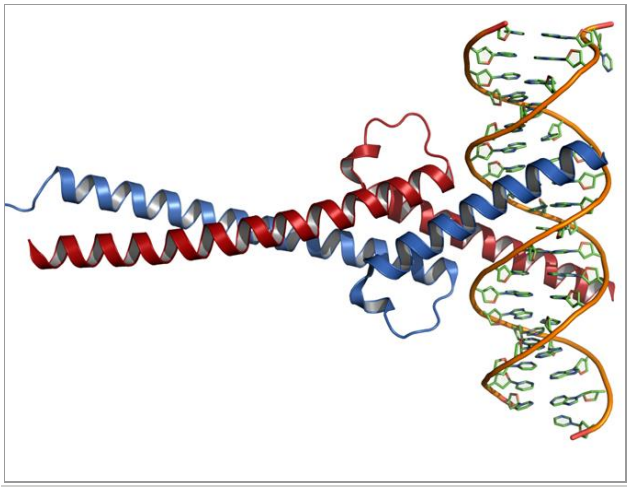
To tackle this problem, first author Dr. Masafumi Toyoshima, principal investigator Dr. Carla Grandori (both of the Human Biology Division) and several colleagues recently set out to identify synthetic lethal interactions with *c-MYC* over-expression, which could potentially contribute to the treatment of *c-MYC*-driven cancers. Synthetic lethality occurs when mutations in two genes lead to cell death. For Toyoshima and co-authors, the core mutational effect of interest was *c-MYC*over-expression, and the desired endpoint involving cell death eventually would be the demise of cancer cells. The primary goal of the research team was to identify the candidate synthetic lethal interaction partners with *c-MYC* aberrant expression, the knockdown of which would avoid the highly toxic side effects of directly targeting *c-MYC* itself.

In the first stage of their successful functional genomics pipeline, Toyoshima *et al.* used a high-throughput siRNA screen involving the knockdown of each of 3,300 genes already known to be part of the druggable genome. They searched for toxic effects on human foreskin fibroblast cells, in which they induced *c-MYC* over-expression using a retroviral expression vector. This led to the identification of 148 candidate synthetic lethal genes. Next, the authors refined their search by

selecting smaller and smaller subsets of candidate genes based on a number of considerations, including: predicted druggability, involvement in cancer pathways, potential side-effects, confirmation of DNA damage and availability of pharmacological inhibitors. Taken together, these criteria narrowed the authors' search to several good candidates for potential use in fighting *MYC*-driven neuroblastoma. The research team focused on one of the candidates (casein kinase 1 epsilon, *CSNK1e*), for which a promising drug had already been developed. Toyoshima *et al.* then demonstrated that*CSNK1e* expression correlates with *N-MYC* amplification in neuroblastoma. In the next step of their screening pipeline, the authors arrived at successful preclinical validation. Using RNAi and small molecule inhibitors, Toyoshima *et al.* confirmed that inhibition of *CSNK1e* halted the growth of *N-MYC*-driven neuroblastoma tumors in mice. Finally, the authors conducted a meta-analysis of human microarray datasets. This revealed that *CSNK1e* expression is positively associated with *N-MYC*amplification in human primary neuroblastoma cells and with poor prognosis for this type of cancer. The meta-analysis further showed that *CSNK1e* expression is statistically associated with *c-MYC* amplification in colon, lung and breast cancers in adults.

In a single impressive body of work, Toyoshima *et al.* identified *CSNK1e* as a promising therapeutic target for certain *MYC*-driven cancers. Moreover, their high-throughput functional genomics pipeline also uncovered a rich therapeutic space of other synthetic lethal targets for potentially treating *MYC*-driven cancer. Of broader significance, the authors demonstrated the future effectiveness of their approach in screening possible therapeutic targets for other oncogenes that were once considered to be undruggable.

Toyoshima M, Howie HL, Imakura M, Walsh RM, Annis JE, Chang AN, Frazier J, Chau BN, Loboda A, Linsley PS, Cleary MA, Park JR, Grandori C. 2012. Functional genomics identifies therapeutic targets for MYC-driven cancer. *Proc Natl Acad Sci USA*109:9545-9550.



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X-ray crystallographic structures of the basic/helix-loop-helix/leucine zipper domains of the c-MYC (red) and MAX (blue) heterodimer bound to its DNA target (the E box hexanucleotide, 5'-CACGTG-3').