Comparative genome analysis identifies the vitamin D receptor gene as a direct target of p53-mediated transcriptional activation

Reo Maruyama, Minoru Toyota, Fumio Aoki, Yasushi Sasaki, Hirofumi Akashi, Hiroaki Mita, Hiromu Suzuki, Kimishige Akino, Kohzoh Imai, Yasuhisa Shinomura and Takashi Tokino

Sapporo Medical University, Sapporo, Japan and SUNY at Stony Brook, New York, NY

p53 is the most frequently mutated tumor suppressor gene in human neoplasia and encodes a transcriptional co-activator. Identification of p53 target genes is therefore key to understanding the role of p53 in tumorigenesis. To identify novel p53 target genes within the human genome, we have used a computational approach focusing on p53 binding sites. The consensus p53 binding sequence consists of two copies of a 10-bp motif (RRRCWWGYYY) separated by 0-12 bases. We first extracted all putative p53 binding sites in the entire human and mouse genomes, and constructed a p53 Response Elements DataBase (PREDB). Next, we used a comparative genomics approach to identify p53 binding sequences conserved in the human and mouse genome. We hypothesized that potential p53 binding sequences that are conserved are more likely to be functional. Using stringent filtering procedures, we finally predicted 101 putative p53 target genes. To assess the validity of the predicted genes by the in silico analysis, their responsiveness to p53 was examined using semi-quantitative RT-PCR and real-time PCR. Of 101 genes analyzed, 32 were up-regulated and 2 were down-regulated by introduction of p53, p63 or p73 in human cancer cells. Among them, we focused on the vitamin D receptor (VDR) gene because vitamin D3 has recently been used for chemoprevention of human tumors. VDR is induced by p53 as well as several other p53 family members, and analysis of chromatin immunoprecipitation showed that p53 protein binds to conserved intronic sequences of the VDR gene in vivo. Introduction of VDR into cells resulted in induction of several genes known to be p53 targets and suppression of colorectal cancer cell growth. In addition, p53 induced VDR target genes in a vitamin D3-dependent manner. Our in silico approach is a powerful method for identification of functional p53 binding sites and p53
target genes that are conserved among humans and other organisms, and for further understanding the function of p53 in tumorigenesis.