The Validation of Anti-Cancer Targets by Clinicopathological study: TGF-β Signaling in Gastric Carcinoma

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The term 'target' carries several connotations in the overall context of drug discovery. The disease-associated proteins are referred to as 'targets' in a broad sense. The process of confirming the deduced specific hypotheses about how the modulation of the expression and the function of the defined proteins by small molecules or biopharmaceuticals could be a route toward improved medicines (to varying degrees of confidence) is usually termed 'target validation'. Currently, target identification is mostly based on the relationship between proteins (or genes) and specific disease, using various –omics and high-throughput technology. However, a simple association between expressed proteins (or genes) and a specific disease does not necessarily validate it as a therapeutic target. In fact, the current drug discovery paradigm of 'gene → protein→ target → hit' is probably oversimplified because the identity of a certain disease has been defined by clinical manifestations and morphological categorization of cells and tissues. The databases created by –omics are necessary but not sufficient, to understand the integrated behavior processes of complex biological systems. Therefore, we should be concerned about a clinicopathological study as early validation tool at the correlation level, and cell-based assays and phenomics - the phenotype identification of genetically engineered mouse, in order to validate therapeutic targets at the level of cause and effect.

The Phenomics of knockout and knock-in mice of a particular gene provide a much clearer link between gene and phenotype. Advances in cell-based assays and cell imaging techniques help solving these problems, which cannot be solved by genetic manipulation. Clinicopathological studies choose 'early-validated target' with the role of the pathogenesis and disease state among targets selected by genomics or proteomics, which may be related to disease, playing a role as a translational research bridging laboratory science and clinical science.

Using immunohistochemistry-based tissue microarray analysis, TGF-β signaling pathway-related molecules (TGFB1/2, TGFBR1 (ALK5), TGFBR2, SMAD1/2/3, SMAD2/3, SMAD4, SMAD7) and downstream targets of TGF-β regulated transcription (CDKN1A ($p21^{CIP1}$), CDKN1B ($p27^{KIP1}$), MYC, CDC25A) and, RELA (p65NF-kB), TP53 were detected in 110 gastric carcinomas, 20 high grade gastric dysplasias, 17 low-grade dysplasias, 41 intestinal metaplasias, 25 chronic atrophic gastritis and 40 samples of normal gastric epithelium. And then the correlation of TGF-β

signaling molecules was evaluated with clinicopathologic parameters in gastric carcinoma.

One of them, SMAD4 expression was shown as a strong prognostic indicator in gastric carcinoma. To investigate the mechanism of SMAD4 downregulation in gastric carcinoma, LOH and 5'-CpG island hypermethylation were firstly performed in 114 gastric carcinoma. Therefore, we confirmed that 114 unselected gastric cancers contained LOH (29%) and 5'-CpG island hypermethylation (5%). The posttranslational regulation of SMAD4 is also essential for downregulation. In 5 gastric cancer cell lines, SMAD4 protein level was detected by western blotting after treatment of MG132 (proteasome inhibitor). The upregulation of SMAD4 was shown in 2 cell lines, SNU484 and AGS. These results suggested that SMAD4 was multiply regulated in gastric carcinoma. Besides SMAD4, many other proteins were shown the correlation with prognosis of gastric cancer. In conclusion, TGF- signaling molecules including SMAD4 have significant correlation with critical clinicopathologic parameters and implicating for the diagnostic and therapeutic potential.