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The Expression and Role of Cylindromatosis in Pancreatic Cancer

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Cancer-related inflammation has been linked to the development of PDAC. Activation of proto-oncogenes and/ or inactivation of tumor suppressors play an important role in the proinflammatory transcriptional program and constitutive production of inflammatory cytokines and chemokines. Previous studies have shown that CYLD exhibits anti-tumorigenic activities in many types of malignancies. CYLD acts as a deubiquitinating enzyme, which inhibits the NF-κB signaling pathway by removing lysine 63-linked polyubiquitin chains from BCL3, a proto-oncogene. This process results in apoptosis and change in cell cycle. However, less is known so far about the possible involvement of CYLD in carcinogenesis of pancreas. Thus the aim of this study was to evaluate the expression pattern of CYLD and its correlation with clinical and pathological parameters and to investigate the potential causal role of CYLD in the development of PDAC.

Histological analysis and molecular assessment were performed in human pancreatic tissue samples. The experiments were based on transient and artificially modified CYLD expression, CYLD plasmid-mediated overexpression and CYLD siRNA-mediated downregulation. The role of CYLD was assessed in human pancreatic cancer cell lines: Colo357 as low CYLD expressor and MiaPaCa2 as high CYLD expressor.

The performed investigations showed downregulated CYLD expression in pancreatic cancer cell lines. In contrast, CYLD expression was increased in PDAC tissues, which can be explained by increased CYLD expression in blood-derived macrophages in stroma-rich PDAC. CYLD expression correlated significantly with PDAC tumor staging: higher expression was observed in higher tumor stage and reduced levels in lower tumor stage. By immunofluorescence CYLD staining in stroma around PanIN lesions and invasive cancer was more intensive when compared to CYLD staining in stroma around normal duct, confirming higher CYLD expression in PDAC tissues. In PanIN-2 lesions and pancreatic cancer cells, CYLD was localized to cytoplasm and did not inhibit nuclear entry of BCL3. We concluded that CYLD underwent change of subcellular localization in an early stage of PDAC

development. Forced upregulation of CYLD resulted in significant inhibition of tumor cell proliferation, increased apoptosis, and changes in cell cycle checkpoints, presumably via NF-κB-dependent pathway. UV-stress induced a transient increase of CYLD in pancreatic cancer cells, indicating that CYLD itself could be regulated by apoptosis. CYLD was recovered by gemcitabine treatment in Colo357, which is associated with wild type p53 and lower basal expression of CYLD. In contrary, CYLD was not altered after gemcitabine treatment in MiaPaCa2 due to its association with mutant p53 and higher basal expression of CYLD. CYLD was upregulated upon hypoxic conditions in five out of eight of the cancer cell lines.

In summary, this study showed that CYLD is downregulated in PDAC and is involved in the complex mechanisms of pancreatic carcinogenesis, such as apoptosis and cell cycle. As current studies are focused on *in vitro* investigation of CYLD effect, further studies should explore CYLD effects *in vivo* and are necessary to identify the exact underlying molecular mechanisms.