The role of MLKL in Liver cancer progression

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Hepatocellular carcinoma (HCC) is the 6th leading cause of cancer-related deaths in the US, with incidence and mortality rates rising faster than any other cancer. Its high mortality, increasing cases linked to the obesity epidemic, and limited treatment options for advanced-stage disease highlight the critical need for further research. Obesity contributes to metabolic-associated steatotic liver disease (MASLD), which can progress to HCC. Chronic inflammation is the key driver of HCC progression and necroptosis is a cell death pathway that promotes inflammation. We found that expression of mixed lineage kinase domain-like pseudokinase (MLKL, the effector molecule in necroptosis pathway) increases in liver in MASLD and inhibiting MLKL reduces HCC in a MASLD-driven HCC mouse model, supporting a role of MLKL in HCC. This study investigates the potential mechanism(s) by which MLKL promotes liver cancer progression. MLKL and its upstream activator, RIPK3, were analyzed in HCC cell lines via Western blotting. MLKL transcripts in mouse liver tumors were measured using q-RT-PCR, while human liver cancer tissue MLKL expression was assessed by immunohistochemistry. In HepG2 cells, MLKL inhibition was achieved using siRNA or the MLKL inhibitor necrosulfonamide (NSA). RNA-sequencing of siMLKL and siControl HepG2 cells, along with cell proliferation assays for siMLKL/siControl and NSA/vehicle-treated HepG2 cells over 96 hours, were also conducted. Our data indicate that MLKL protein expression is elevated in human liver cancer cell lines, with RIPK3 absent in all. MASLD-driven HCC mice also showed increased MLKL levels in both tumor and non-tumor liver tissues compared to controls. Similarly, human liver cancer tissues exhibited significantly higher MLKL protein than adjacent normal tissue. RNA-seq revealed that cell cycle regulation was a major downregulated pathway upon MLKL depletion in HepG2 cells. Both genetic and pharmacological MLKL inhibition reduced HepG2 cell proliferation. In summary, the absence of RIPK3 suggests that MLKL-mediated necroptosis is inactive in liver cancer cells. Thus, our study identified a novel non-necroptotic role of MLKL in cell proliferation, potentially via cell cycle regulation. This highlights MLKL as a promising therapeutic target in combating HCC progression.

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