KRUPPEL-LIKE FACTOR 4 SUPPRESSES NEUROBLASTOMA GROWTH BY PROMOTING SMOOTH-MUSCLE DIFFERENTIATION

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Neuroblastoma (NB) is an embryonic tumor and possesses a unique propensity to exhibit either a spontaneous regression or an unrestrained growth. Growing evidence suggests that NB comprises heterogeneous populations of improperly differentiated neural crest cells and a small subset of NB cells behaves as stem cells. Commitment of NB stem cells to the fibromuscular lineage may give a favorable outcome, while to the neuronal lineage results in a malignant tumor progression. Krüppel like factor 4 (KLF4) is one of the key reprogramming factors. Intriguingly, it also possesses paradoxical functions in cancers, either as an oncogene or tumor suppressor dependent on cell context. In this study, we elucidated the roles of KLF4 in the lineage determination of NB stem cells and tumor progression. Quantitative RT-PCR showed that loss of KLF4 expression was frequently found in the high-stage NB (Stages III and IV). In particular with the high-risk factors such as age of patient >1 year, N-myc amplification and low TrkA expression, the decreased expression of KLF4 was significantly associated with an unfavorable NB outcome. Subsequent targeted down-regulation studies using a NB cell line (SK-N-SH and Be(2)-C) directly demonstrated that reduced KLF4 expression favors the growth of NB cells and tumorigenesis. In concordance with this, overexpression of KLF4 profoundly suppressed proliferation and induced apoptosis of NB cells (SH-SY-5Y). At the molecular level, KLF4 directly up-regulated the cell-cycle inhibitor protein p21CIP and induced cell cycle arrest and cell death. In addition, KLF4 overexpressing cells have lost their neuroblastic phenotypes, they were epithelial-like, strongly substrate-adherent, expressing smooth muscle marker and became non-tumorigenic. Moreover, KLF4 knockdown clones were not able to committed to fibromuscular lineage, suggesting that KLF4 expression is crucial for lineage determination of NB stem cells. Collectively our work showed that decreased KLF4 expression is associated with poor disease outcome and KLF4 can directly mediate the growth and lineage determination of NB cells.