Notch Signaling–related Therapeutic Strategies With Novel Drugs in Neuroblastoma Spheroids

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Summary: Neuroblastoma is a severe pediatric tumor characterized by poor prognosis. Identification of novel molecular targets and diversion of investigations on new drug trials is mandatory for cancer therapy. In this study, vinorelbine tartrate, lithium chloride, clomipramine, and medroxyprogesterone acetate are used for the possible new treatment modalities in neuroblastoma cells. Notch and c-kit are novel molecules in cancer research, and Notch pathway is one of the emerging molecules in the neuroblastoma pathogenesis. Cytotoxic effects of these drugs at different time points, with different doses were studied in the SH-SY5Y human neuroblastoma cell line. Analysis of Notch and c-kit signaling with immunohistochemistry were constituted in multicellular tumor spheroids, and morphologic investigation was performed for digital imaging of cancer stem cells (CSCs) with electron microscopy. Size kinetics of spheroids was also determined after drug treatment. Results showed that all drugs were cytotoxic for neuroblastoma cells. Yet, this cytotoxic action did not correlate with the inhibitory effects in cell signaling. Neuroblastoma spheroids showed increased immunoreactivity of Notch signaling and c-kit. Altered ultrastructural CSCs morphology was observed after clomipramine and medroxyprogesterone acetate treatment compared with other drugs. Lithium chloride showed cellular membrane destruction for both CSCs and the remaining population. In this study, independent effects of cytotoxicity in tumor cells with respect to CSCs were determined. Redundant cells, which are the bulk population in tumor a compound, destroyed with therapy, were neither a target for treatment nor a remarkable investigation of cancer.

Key Words: vinorelbine tartrate, lithium chloride, clomipramine, medroxyprogesterone acetate, Notch, c-kit, neuroblastoma, cancer stem cell

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Recently, scientists have witnessed a transition from a state of assumptions and hypothetical derivations regarding the role of stem cells in the onset and progression of cancer to the establishment of actual experimental proof in the isolation and identification of "cancer stem cells" (CSCs) for several types of tumours.[1]

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The realization of CSCs in tumors has changed our view of carcinogenesis and chemotherapy as the existence of CSCs is mainly responsible from tumor relapse, metastases, and overall cancer survival. Self-renewal and high clonogenic potential are characteristics shared by both normal stem cells and CSCs.[2] Among similar phenotypes of normal cells and CSCs, to identify and isolate CSCs endeavor a challenge for novel drug development and treatment strategies.

Neuroblastoma is the most common pediatric abdominal solid tumor, and this aggressive embryonic malignancy originates from neural crest. Although mechanical cell separation is possible for identification of CSCs in neuroblastoma, some difficulties still arise because of the unknown surface markers of cancerous cell population. In current treatment protocols, including high-dose chemotherapy with autologous stem cell transplantation, radiation, and surgery, most of the high-risk patients go into remission, whereas the majority relapse and succumb to therapy-resistant tumors.[3]

Deregulation of embryonic-signaling pathways in normal stem cells constitutes CSC properties.[4] Enrichment of CSCs from established variety of solid tumor cell lines developed as a 3-dimensional (3D) cell culture. 3D spheroid model is a new technique for propagation of cells in vitro using serum-free medium and cultured low-adherence conditions.[5] This technique has been applied in this study for investigation of novel drug applications, and Notch signaling was focused for the response. Because CSCs are resistant to chemotherapy and there were many attempts to target CSCs by modifications in alternative-signaling pathways.

Notch signaling plays an important role during development of the embryonic and adult tissues and homeostasis of adult self-renewing organs. In embryogenesis, Notch regulates the cell-lineage decisions and modulates the differentiated state in mature cells. This pathway also attends the regulation of many fundamental processes such as stem cell maintenance, proliferation, and differentiation.[6–8] During neurogenesis, Notch effects neural sympathetic precursor cells by inhibition of neuronal differentiation and maintains proliferative stage of these precursor cells.[9,10] Hereby, Notch signaling plays a critical role in the progression of several cancers through the regulation of the main cellular functions associated with tumor genesis such as proliferation, angiogenesis, and cell migration.[11,12] In neuroblastoma, Notch is an emerging molecule and Notch pathway is correlated with neuroblastoma pathogenesis, particularly related to its key role in neural embryonic development.[13] Recent studies revealed that Notch pathway is one of the most intensively studied putative therapeutic targets in CSCs, and several investigational Notch inhibitors are developed and some are