THE ROLE OF NON-NEURONAL CHOLINERGIC COMPONENTS IN LEUKEMOGENESIS

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ABSTRACT

The non-neuronal cholinergic system (NNCS) and its components have been shown to play a role in hematopoietic differentiation. I hypothesized that alteration of NNCS could also play a role in mediating leukemogenesis. The expression of cholinergic components in leukemic cell lines was determined by Western blotting and in normal leukocyte subsets by flow cytometry. The results of this study showed a heterogeneous expression of choline acetyltransferase (ChAT), acetylcholinesterase (AChE), choline transporter (CHT), M3 muscarinic acetylcholine receptor (M3-mAChR) and α7 nicotinic acetylcholine receptor (α7-nAChR). Within normal leukocyte subsets, neutrophils exhibited the highest AChE (degrading acetylcholine enzyme) expression as compared to monocytes and unfractionated lymphocytes, and B lymphocytes exhibited a higher AChE expression compared to NK cells and T lymphocytes. Moreover, malignant B lymphocytes ($CD19^+CD5^+$) showed a significantly decreased expression as compared with normal B lymphocytes ($CD19^+CD5^-$) in B-cell chronic lymphocytic leukemia (B-CLL) patients ($P<0.024$). I then evaluated NNCS's role in the differentiation of the human NB-4 acute promyelocytic leukemia cell line and found a dramatic induction of M3-mAChR after all-trans retinoic acid (ATRA) treatment ($p<0.0001$). Adding the cholinergic agonist, carbachol, to ATRA treatment led to an increase of CD11b, a granulocytic differentiation marker compared with ATRA treatment alone ($p<0.05$), indicating that cholinergic activation enhanced ATRA in inducing NB-4 maturation. The combination of carbachol and ATRA treatment resulted in decreased viability as determined by XTT cell viability assay and increased cleaved caspase-3 expression when compared with ATRA treatment alone ($p<0.05$). However, this combination did not cause poly (ADP-ribose) polymerase (PARP) cleavage, indicating that carbachol treatment led to apoptosis via caspase-3 activation but not PARP activation. Overall, NB-4 cells expressed M3-mAChR in a differentiation-dependent manner and cholinergic stimulation induced maturation and apoptosis of ATRA-induced differentiated NB-4 cells. Furthermore, the investigation of the genomic variations of NNCS components was delineated by performing whole exome sequencing (WES), resulting in an innovative bioinformatics workflow. In a run-in set of experiments, a variation in the M5-muscarinic acetylcholine receptor (M5-mAChR) in a T-acute lymphoblastic leukemia (T-ALL) patient was detected and predicted to regulate the muscarinic signaling pathway, cell proliferation, and dopamine transport, although variations of other NNCS components were not detected in other leukemia subtypes. In conclusion, NNCS can mediate leukemogenesis via its components and cholinergic stimulation can enhance the development of ATRA-induced differentiation of NB-4 leukemia cells. These findings suggest a potentially novel therapeutic regimen utilizing cholinergic stimulation in standard differentiation-inducing regimens in acute leukemia.

KEY WORDS: NON-NEURONAL CHOLINERGIC COMPONENTS / LEUKEMOGENESIS / ACETYLCHOLINESTERASE / DIFFERENTIATION / WHOLE EXOME SEQUENCING

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