NK Cell Therapy of Cancer : Enhancing Function by Hypoxic Education

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NK cells provide the first line of defense against tumor and viruses, without the requirement for prolonged pre-activation. However, anti-tumor function of NK cells is progressively dampened in the terminal stages of cancer patients, largely associated with the tumor-induced immune evasion mechanism. Hypoxic tumor environment provides once such mechanism providing NK cell tolerance, and become a potential therapeutic problem to overcome in the patients of solid cancer for NK immunotherapy. To better understand the effect of hypoxia on NK cells, we assessed the anti-tumor function of NK cells in response to decreasing concentrations of O2 pressure. As expected, NK cells exposed to severe hypoxia, 0.5% O2, demonstrated diminished cytotoxicity and IFNg against A375 melanoma targets and failed to expand to a larger number ex vivo. However, NK cells incubated in mild hypoxia of 1.5% O2 showed slightly better CTL activity than normoxic condition, but still failed to proliferate. These data explain why there are so few NK cells within resected solid tumor tissues. Since CTL killing activity is elevated in NK cells cultured in 1.5% hypoxia, we hypothesized that if we pre-activate NK cells in normoxia prior to switching to hypoxia, we could educate (or license) NK cells to function and proliferate within hypoxic tumor microenvironment. To test this hypothesis, we started culturing NK cells in normoxia then transferred them into 1.5% hypoxia chamber at day 3, 5, 9. Our results showed that NK cells switched to day 5 or 9 were expanded to a much higher number and mounted increased anti-tumor effector function, when CTL reaction was performed both in normoxic and hypoxic conditions. But those switched to 1.5% hypoxia chamber after 3 days did not proliferate, indicating that NK cells need O2 for initial priming. The hypoxia-exposed NK cells demonstrate upregulation of major activating NK cell receptors, NCRs and NKG2D receptors including STAT3, while inhibiting apoptosis during ex-vivo expansion. Microarray analysis results revealed that AK3L1, PFKFB4, MT1G, BNIP3 genes were elevated and BTLA, KLRG1 genes were downregulated in hypoxia-exposed NK cells, providing the basis for the increased NK effector function and survival. These data highlight the need for ex-vivo activated NK cell therapy, and further suggest that NK cell education within hypoxic environment during expansion can indeed facilitate the proliferation and function of NK cells that can overcome NK cell intolerance in the patients undergoing NK cell immunotherapy.