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BEYOND NETS: DNASE I TREATMENT SHOWS IMMUNOSTIMULATORY EFFECTS AND MODIFIES NEUTROPHIL PHENOTYPE AND FUNCTIONALITY IN TUMOR-BEARING MICE *

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Abstract

DNase I is a promising anti-tumor agent degrading neutrophil extracellular traps (NETs). However, its effect on the immune system beyond NET degradation is not investigated. In this study, we show that DNase I treatment in mice bearing lymphosarcoma RLS_{40} enhances the anti-tumor neutrophil phenotype, reduces neutrophil capacity to produce NETs, and induces immune activation. These findings suggest a potential role for DNase I in future anti-cancer therapies.

Recently, neutrophils have been identified as important players in the tumor microenvironment (TME). They were shown to acquire antitumor or protumor phenotypes in the TME. Moreover, neutrophils can secrete neutrophil extracellular traps (NETs), which support tumor growth and metastasis. DNase I treatment has been shown to reduce tumor growth and aggressiveness, mainly by degrading tumorigenic cell-free DNA and NETs. In this study, we investigated the effect of DNase I treatment on the neutrophil population and the overall immune response.

A murine model of chemotherapy-resistant lymphosarcoma (RLS_{40}) was established, and mice were administered DNase I (100 U/ mouse) or saline buffer (control) for 5 days per week, with the mice being left untreated for 2 days, over the course of 3 weeks. On day 21, the mice were divided into 2 groups in accordance with tumor node size (RLS_{40}^{High} > 1 cm^3, RLS_{40}^{Low} < 0.1 cm^3). Histological analysis of tumors and thymus were performed, and splenic neutrophils were isolated and characterized using RT-qPCR, flow cytometry and florescent microscopy.

DNase I treatment exhibited immunostimulatory effects, as evidenced by an increased thymus cortex-to-medulla ratio and enhanced macrophage infiltration in the thymus and tumors. In comparison with the RLS_{40}^{High} group, neutrophils from the RLS_{40}^{Low} group exhibited an antitumor profile with higher *Icam*, *Tnfa*, and *Cd274* gene expression. Treatment with DNase I enhanced the observed anti-tumor features with a significant elevation in the expression of the above-mentioned genes and a decrease in the expression of the immunosuppressive genes *Ccl17* and *Il10*. Flow cytometric analysis showed an increase in the percentage of anti-tumor neutrophils in the RLS_{40}^{Low} group after DNase I treatment, with an increase in the PD-L1$^+$, ICAM-1$^+$, FAS$^+$ and IFNɣR1$^+$ populations. The most interesting result was the ability of DNase I to reduce the capacity of neutrophils to produce NETs after chemical activation in vitro with calcium ionophore A23187. Neutrophils isolated from RLS_{40}^{High}-bearing mice were characterized with higher NET formation percentage in comparison to RLS_{40}^{Low} group. Neutrophils isolated from DNase I treatment groups were characterized by reduced ability to secret NETs.

In conclusion, in tumor-bearing mice DNase I treatment shows immunostimulatory effects and modifies neutrophil phenotype and functionality. These findings highlight the potential of DNase I as a future anti-cancer therapy.

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