Neutrophil extracellular traps (NETs) – novel target for drug delivery to various pathological sites

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Neutrophil extracellular traps (NETs) are composed of processed chromatin bound to granular and selected cytoplasmic proteins and released by neutrophils. NETs consist of smooth filaments composed of stacked nucleosomes. Fully hydrated NETs have a cloud-like appearance and occupy a space 10–15-fold larger than the volume of the cells they originate from. DNases are the enzymes that cleave extracellular DNA including NETs. The earliest report on NETs is dated to 1996, however the story on a crucial role of NETs in immunity, specifically in innate immunity, started in 2004 when their protection against microbial invaders was demonstrated. Intense later studies followed where the presence of disease site NETs and their major role in innate immunity of the disease sites was validated for three major disease groups, namely infectious inflammations (bacterial, viral, etc.), sterile inflammations, (I/R, like infarctions, etc.), and tumors. Together with their protective role in microbial infections, NETs are involved in multiple pathological processes and represent key events in a variety of pathologies including cancer, autoimmunity, and cardiovascular disease. Sites of NETs concentration are dangerous for the host if the process of NETs formation becomes chronic or the mechanism of NETs removal does not work. NETosis has been linked to the development of thrombosis, periodontitis, cystic fibrosis, type 2 diabetes, COVID-19 or rheumatoid arthritis as well as cancer progression. In numerous studies causative association of NETs with disease site inflammation and disease severity was demonstrated and multiple NET-suppressive therapies are in preclinical and clinical studies in connection with all three aforementioned disease group.

Thus, the destruction of NETs in is of primary significance in many pathologies. On the other hand, NETs may serve as an ideal universal target to specifically deliver drugs into disease zone in multiple pathologies, which was not explored so far. Currently, the major targets of vehicles used in targeted delivery are certain cell surface antigens specific to a disease site, or some extracellular matrix antigens, specific to the disease site. However, these targets differ for different pathologies and require different vehicles for targeting, while NETs could serve as a universal target. It is specifically related to NET chromatin, which is an obligatory constituent of every NET, contrary to other NET components, like various neutrophil proteins decorating NET chromatin.

Monoclonal antibody 2C5 was discovered by us as a nuclear-reactive autoantibody from the B–cell repertoire of normal aged mice. This Ab was shown to have a nucleosome-restricted specificity and used intially to target cancer cells via the cancer cell surface-bound nucleosomes. Recently, mAb 2C5 has been proven to effectively recognize NETs of different etiologies, including compacted NETs.

We confirmed the specificity of 2C5 toward NETs by ELISA, which showed that it binds to NETs with the specificity like that for purified nucleohistone substrate. We further utilized that feature to create two delivery systems (liposomes and micelles) in particular for DNAse I enzyme to destroy NETs, and assume that these drug delivery vehicles and also (or simultaneously with DNase) co-loaded with drugs for the treatment of the primary disease accompanied by NETs formation, such as thrombolytic enzyme for the treatment of thrombosis or chemotherapeutics for treating cancer.

In our opinion, NETs may be considered as a universal target in many diseases and mAb 2C5-modified delivery systems can serve as a universal platform for targeting multiple pathologies.