Characterization of Extracellular DNA Released from HeLa Cells During Apoptosis and Necrosis

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Abstract

All extracellular media contains DNA; however, how DNA is released from cells is poorly understood. In our lab, we sought to create new methods to characterize and quantify the DNA released from dying cells in a simple mammalian cell culture system. We measured concentrations of free exDNA and vesicular (or membrane protected), exDNA during apoptosis or necrosis induced by different stimuli. Our results suggest that most of the exDNA is free as opposed to membrane-protected, but the proportions vary between different types of cell death.

Introduction

The existence of extracellular nucleic acids has been known since 1948, when they were first discovered in blood plasma (1). However, little is known about the release of extracellular DNA (exDNA) and extracellular RNA and how this process varies between different types of cell death (2). For example it is unknown what proportion of exDNA is membrane bound (in apoptotic bodies or other vesicles) vs. free (3). Understanding how mammalian cells release exDNA during different physiological processes is important for understanding how the immune system differentiates self (dying cells) from non-self (viral or bacterial DNA). It has been shown, for example, that exDNA is involved in some inflammatory and autoimmune diseases such as Lupus (4). Furthermore, there are important implications for understanding exDNA in cancer diagnostics, as exDNA is currently being used to develop “liquid biopsies”: non-invasive cancer diagnostics (5).

Studying exDNA in human biological fluids such as blood plasma is complicated due to the fact that many different cells release a large variety of macromolecules into these fluids. We decided to study exDNA release in a much simpler in vitro cell culture system where we could precisely control the processes that cells undergo. We induced apoptosis and necrosis in HeLa human cancer cells and then collected DNA from the cell culture media. We then characterized this DNA to investigate questions such as whether it is membrane bound or free.

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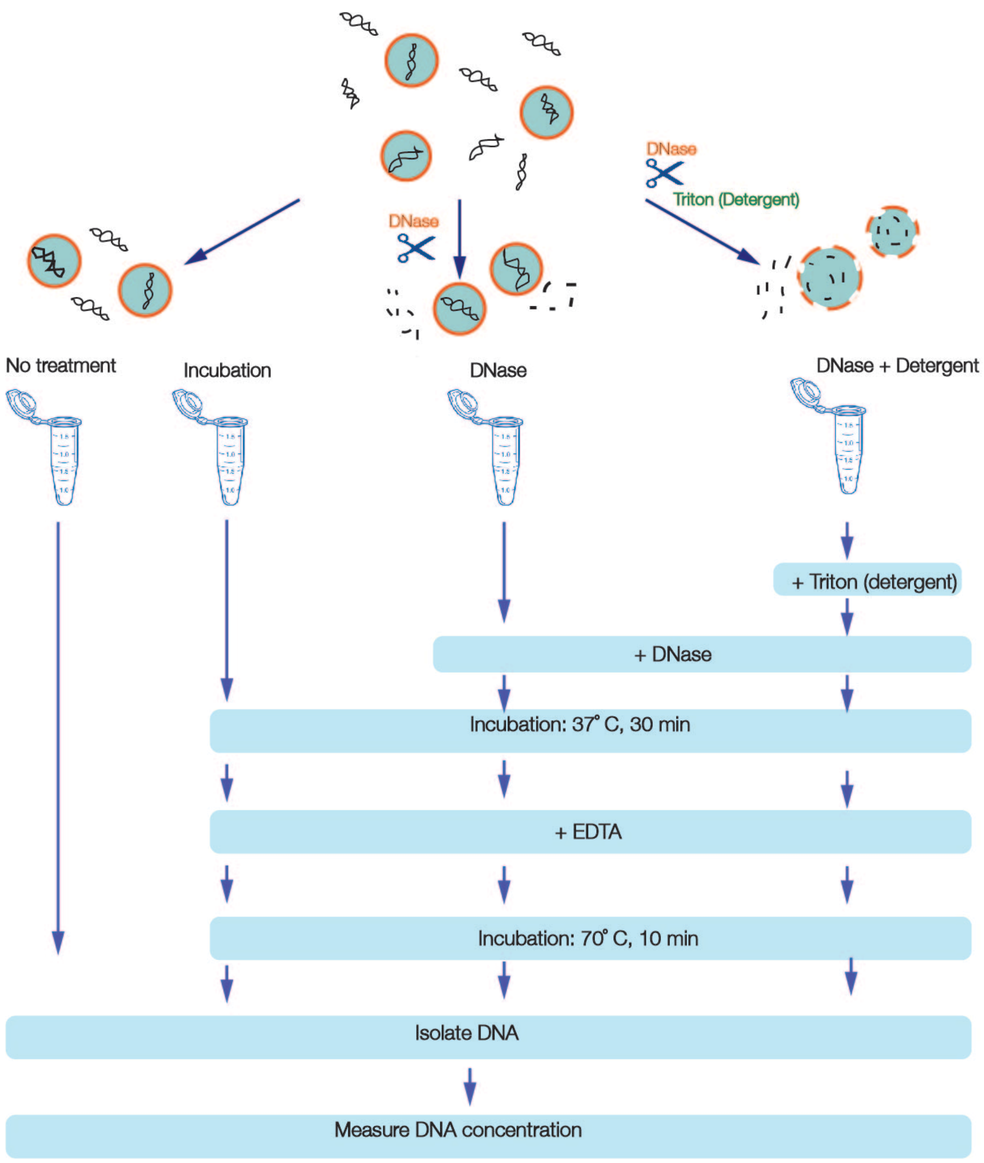
Materials and Methods

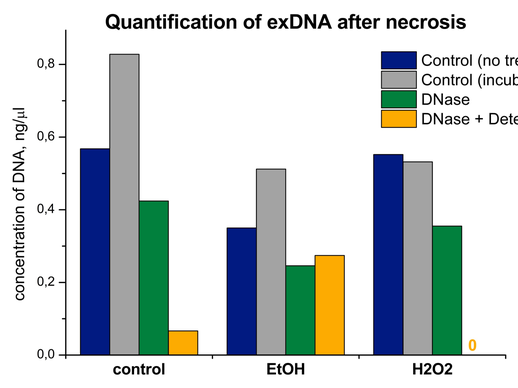
HeLa cells were grown in medium consisting of DMEM (high glucose GlutaMAX™ Supplement) with 10% Fetal Bovine Serum (FBS) and 1% Penicillin Streptomycin (Gibco). 70% Ethanol and hydrogen peroxide (H_2O_2), were used to induce necrosis while staurosporine (SSR) (Sigma Aldrich) was used to induce apoptosis. DNA in 10 mL cell culture media was isolated using Vivaspin 20 5 kDa cutoff spin-filters. The exDNA in 8mL Fetal Bovine Serum (Gibco) was isolated using GenElute™ Plasma/Serum Cell-Free Circulating DNA Purification Midi Kit. Cell culture exDNA was further separated with the use of either Quick DNA extraction kit (Zymo Research) protocol or GenElute™ Plasma/Serum Cell-Free Circulating DNA Purification Midi Kit. We split the obtained DNA mixture into four samples. One negative control (no treatment) received only lysis buffer. The second sample received Turbo DNase buffer (ThermoFisher), EDTA at final concentration of 15 μM (ThermoFisher), and lysis buffer. The third sample received 5 μL DNase, EDTA, and a lysis buffer. The last sample received DNase and 3% triton X (Sigma Aldrich). After 30 minutes of incubation at 37C, EDTA was added to each sample and moved to 70C for 10 minutes. Concentrations of exDNA were measured with a Qubit High Sensitivity dsDNA kit (ThermoFisher).

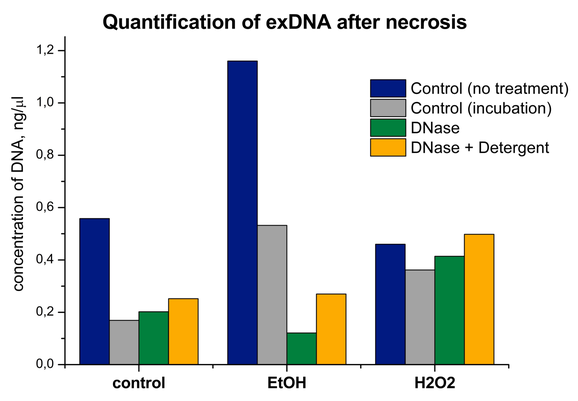


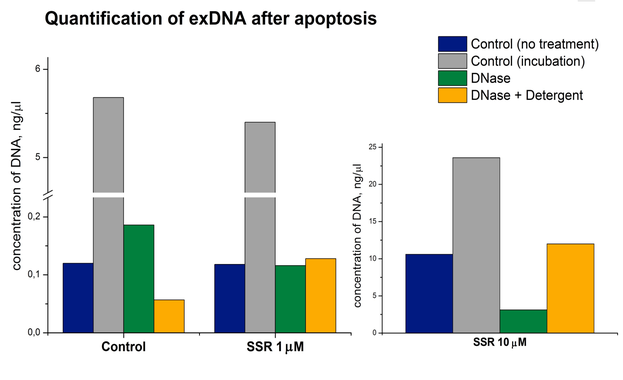
Media collected from cells was separated into 4 parts and underwent no treatment, EDTA treatment and incubation, DNase and EDTA treatment or detergent, DNase and EDTA treatment. Then DNA was isolated and its concentration was quantified by Qubit.

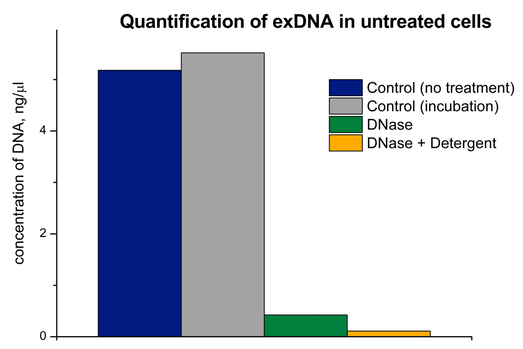
Results

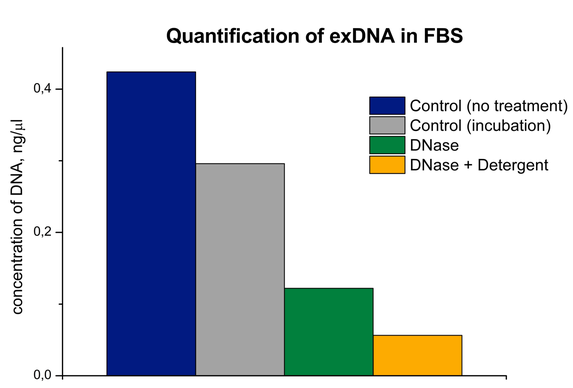
Media isolated from dying cells was treated with the conditions depicted below. DNA was isolated and quantified by Qubit.

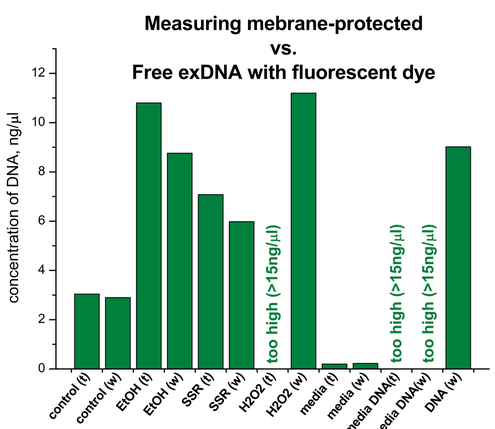












Treatments: detergent (t) and water (w)

Conclusions

The main goal of our laboratory is to isolate and characterize the DNA secreted during apoptosis and necrosis. How does DNA exist in the extracellular space – is it packaged into membrane-bound apoptotic bodies or is it free? We devised methods to study this question based on treatment of free DNA with DNase and comparison with DNA after treatment with both DNase and a membrane-disrupting detergent (Triton X). Our results indicate that most exDNA in cell culture media is free rather than protected membrane, but some is indeed in vesicles (such as apoptotic bodies). We also found this to be the case when we induced necrosis, although more replicates are needed to figure out the precise proportion. We were also able to apply our methods to the isolation of DNA from Fetal Bovine Serum (as a more accessible model for human serum) and showed that most exDNA in FBS is also free rather than membrane bound. Future studies will characterize other features of exDNA during apoptosis and necrosis such as DNA fragmentation and exDNA-bound proteins. It would also be interesting to investigate the differences in immunogenicity of exDNA after induction of necrosis and apoptosis, as well as the differences on immune cell stimulation between membrane-bound and free DNA.