

Investigation of Apoptosis and Necrosis in HeLa Cells

Max Bahdanovich^{1,5}, Roman Burlutskiy^{1,5}, Anna Culinscaia^{1,5}, Sasha Denisova^{1,5}, Karolina Dubovskaya^{1,5}, Igor Iziumov^{1,5}, Kate Lyublinskaya^{1,5}, David Noyvert^{1,5}, Vlada Kostitsyna¹, Yulia Ivanova^{1,4}, Tiffany Amariuta^{1,2}, Dima Ter-Ovanesyan^{1,3}

¹Laboratory of Extracellular Nucleic Acids, School of Molecular and Theoretical Biology
²Department of Biomedical Informatics, Harvard Medical School, Boston, MA, USA
³Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, USA
⁴Skoltech Institute of Science and Technology, Moscow, Russia
⁵These authors have equally contributed to this work



Abstract

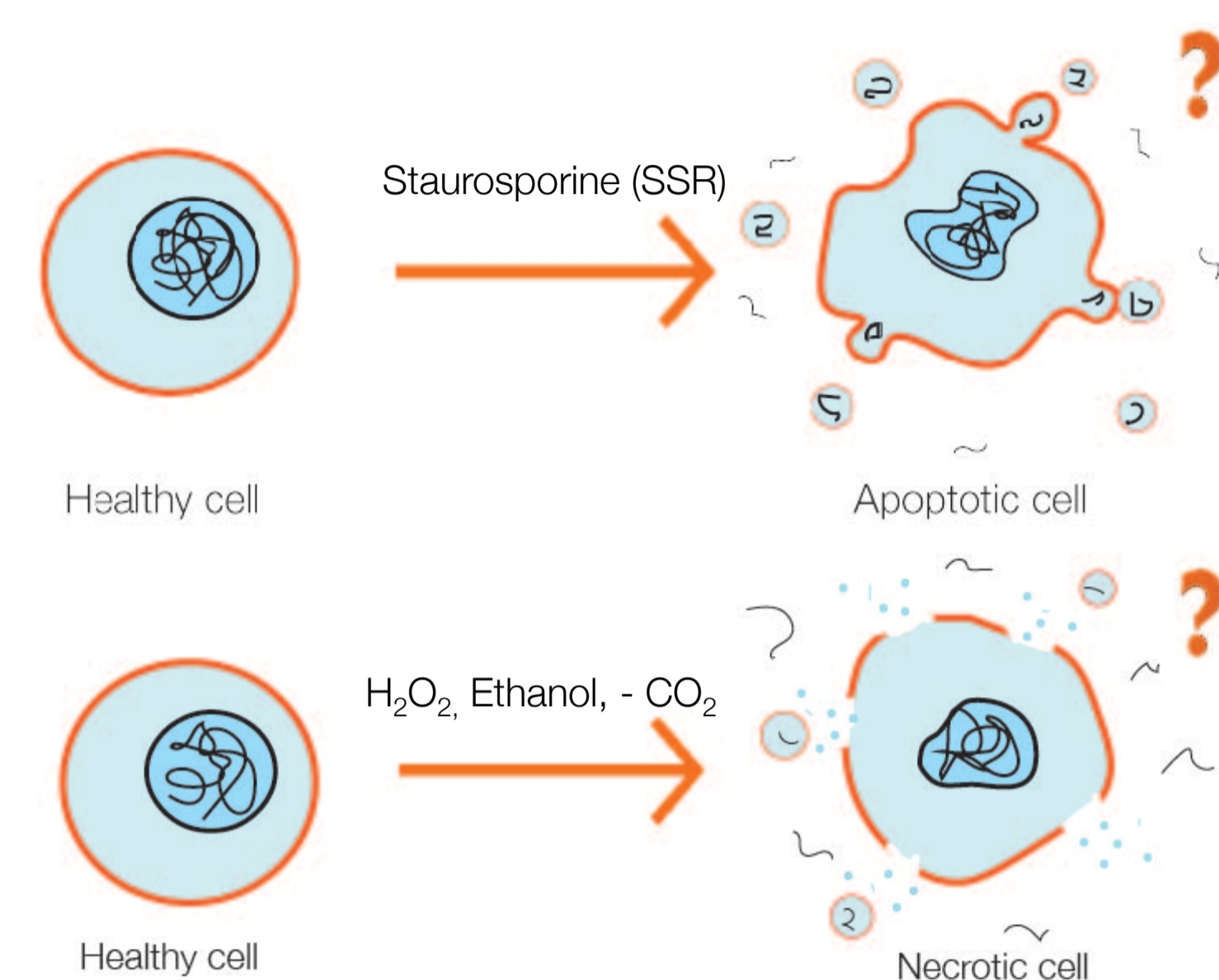
Extracellular nucleic acids are DNA and RNA found outside of cells. We investigated the dynamics of cell death in order to study extracellular nucleic acids. In our research, we induced apoptosis by staurosporine (SSR) and necrosis by ethanol or hydrogen peroxide in a cell culture system of HeLa cancer cells. We optimized drug concentrations and measured cell viability at different time points. We also ran gel electrophoresis on apoptotic and necrotic cellular DNA to study its fragmentation and cell morphology during cell death by microscopy. The determined conditions were used to study extracellular DNA (exDNA) for all of our studies.

Introduction

In order to study extracellular DNA (exDNA) during different types of cell death, we first had to characterize the dynamics of cell death in our system. We utilized the HeLa human cancer cell line, due to its robustness and widespread use, to study exDNA during apoptosis and necrosis.

Apoptosis is a mechanism of programmed cell death. Necrosis, on the other hand, is a less well-defined process caused by external factors (1). We had to optimize the conditions for inducing apoptosis and necrosis. We investigated cell viability at different time points after cell death for different stress-inducing stimuli: staurosporine (a drug that blocks kinase activity) for inducing apoptosis (2), and ethanol or hydrogen peroxide (H₂O₂) for inducing necrosis. Determining these time points were important to ensure sufficient cell death and also minimize the possibility that DNA may degrade over time in cell culture media (3). In order to minimize this degradation, we sought to select time points for exDNA isolation shortly after cell death. We further characterized DNA fragmentation in cells during apoptosis and necrosis, as well as cell and nucleus morphology by fluorescence microscopy.

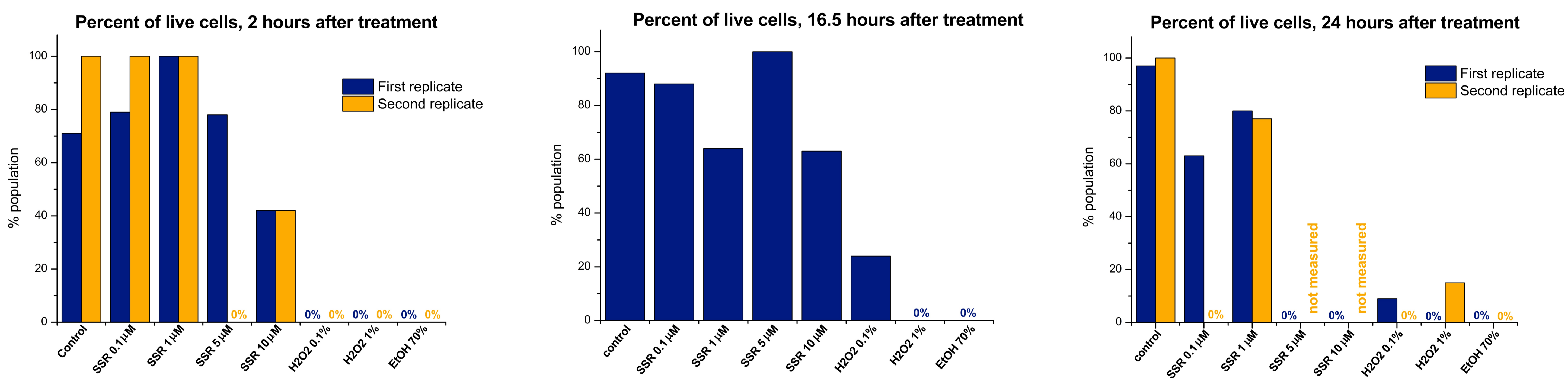
1. Taylor, R. C., Cullen, S. P. & Martin, S. J. Apoptosis: controlled demolition at the cellular level. *Nat. Rev. Mol. Cell Biol.* **9**, 231–241 (2008)., 2. Belmokhtar, C. A., Hillion, J. & Ségat-Bendirdjian, E. Staurosporine induces apoptosis through both caspase-dependent and caspase-independent mechanisms. *Oncogene* **20**, 3354–3362 (2001)., 3. Beyer, C. *et al.* The extracellular release of DNA and HMGB1 from Jurkat T cells during in vitro necrotic cell death. *Innate Immun.* **18**, 727–37 (2012).



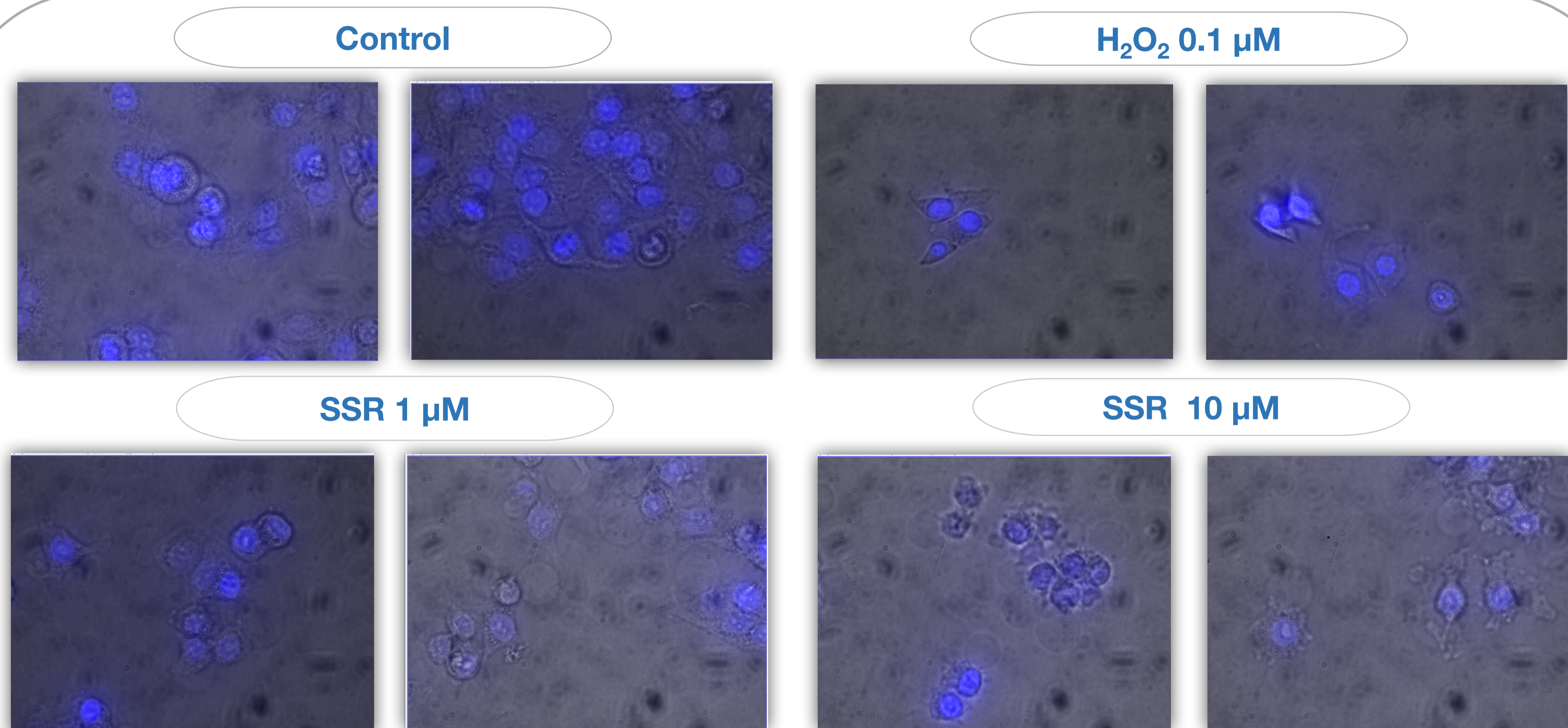
Materials and Methods

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 (H₂O₂), were used to induce necrosis while staurosporine (SSR) (Sigma Aldrich) was used to induce apoptosis. TC20 Automated Cell Counter (Biorad) was used to quantify cell counts and viability using a size cutoff of 10–20 μm. For the microscopy and viability experiments, 6 well plates for cell culture (Falcon) were used, and for all other experiments 10 cm diameter dishes for cell culture (Falcon) were used. In order to run DNA electrophoresis, we used a 2% agarose gel with a 50 bp ladder (New England Biolabs). Microscopy was performed after fixing the cells with 4% paraformaldehyde and staining with DAPI on a Zeiss epifluorescent microscope.

Results

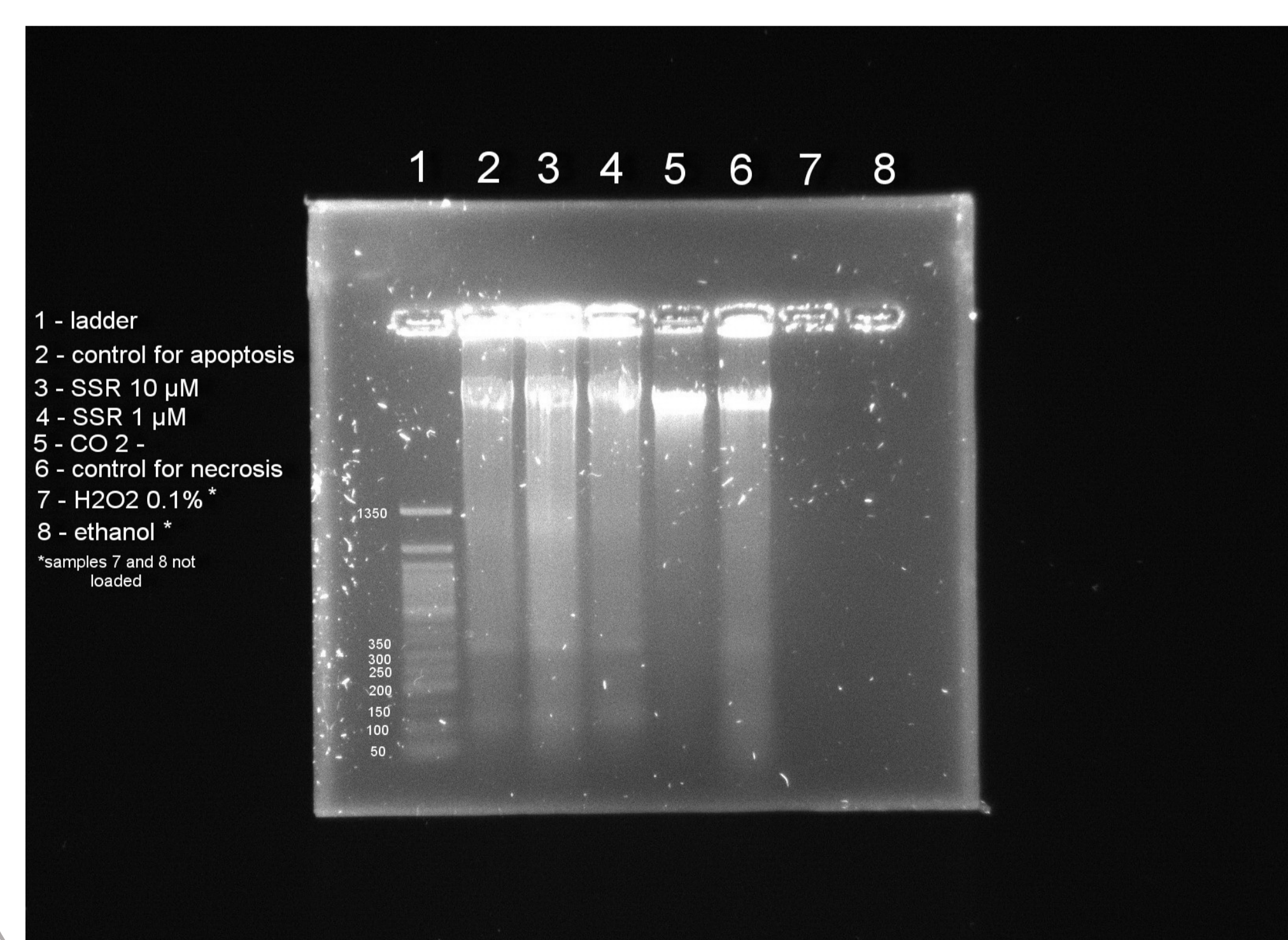


Percent of live cells measured by the automatic cell counter within X hours of treatment by SSR at concentrations 0.1 μM, 1 μM, 5 μM, and 10 μM, H₂O₂ at 0.1% and 1% or 70% ethanol. Control cells were untreated. Two colors refer to two replicates.



Fluorescence microscopy in fixed cells 1 hour after treatment. Blue indicates DAPI nuclear stain.

Gel Electrophoresis



Cellular DNA after induction of cell death.

Conclusions

We were able to optimize the induction of apoptosis and necrosis to make the majority of cells die in the shortest time. Among ethanol (70%), hydrogen peroxide of various concentrations (0.1% and 1%) and Staurosporine of varying degrees of dilution (0.1 μM, 1 μM, 5 μM, 10 μM), we picked concentrations of treatments that induced complete or near-complete cell death. For apoptosis by Staurosporine, we chose a 24-hour incubation period, which was the shortest time resulting in a majority of cells dying (as measured by Trypan Blue). We found that necrosis, on the other hand, is induced much more quickly than apoptosis, and we thus decided to choose a time point of one hour after induction of necrosis. For the DNA fragmentation analysis, we did not find significant differences between the DNA of cells where apoptosis was induced and control cells. This may be because we only chose one time point for necrosis and one for apoptosis. There were some changes in cell morphology after different types of cell death but future experiments should include more time points and also membrane dyes to better characterize changes in membrane morphology.