

**STUDY OF PROTEIN ACTIVITY IN HELA CELLS
SUBJECTED TO NANOSECOND PULSED
ELECTRIC FIELDS***

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Biological applications of pulsed electric fields (PEF) have been discussed in the last decade. Schoenbach et al have investigated the biological activity of various kinds of cells subjected to nanosecond pulsed electric fields (nsPEF). They reported nsPEF induces the increase in intracellular Ca²⁺, DNA fragmentation, activation and inactivation of proteins related to apoptosis¹. Moreover, they successfully reduced melanoma on mice by the application of nsPEF. Here, we discuss the reaction pathway of apoptosis in HeLa S3 cells subjected to nsPEF. We investigated the activation of several kinds of proteins related to mitochondria and endoplasmic reticulum (ER) to understand how nsPEF activate apoptosis in HeLa cells by means of polymerase chain reaction (PCR) method, real-time PCR method and antibody. HeLa cells were subjected to 100 ns-long PEFs with various parameters in terms of field strength (< 20 kV/cm), frequency (< 250 pulses per second) and shot number (<100). mRNA expression in the cells were analyzed at 2 and 6 hours after the pulsing. We found that the application of nsPEF induces the activation of caspase3, proteins activated in the late phase of apoptosis. The activation of mitochondria related proteins, Bcl-2 family has not been detected so far. Presently we are investigating the activity of calcium dependent proteins, calpains and mitochondria independent proteins, caspase12. We will figure out apoptosis pathway of in HeLa cells on the basis of all of the investigation.

1. R. Nuccitelli, X. Chen, A. G. Pakhomov, W. H. Baldwin, S. Sheikh, J. L. Pomicter, W. Ren, C. Osgood, R. J. Swanson, J. F. Kolb, S. J. Beebe, K. H. Schoenbach, *Int. J. Cancer* Vol. 125, pp. 438–445 (2009)

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