Quantification of apoptosis and detection of HSP70 and COX-1 expression in tumor cells during Ehrlich ascites tumor growth

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Cell death by apoptosis is a common event during tumor progression, especially when nutrients, oxygen and space are lacking. But some tumor cell types are relatively resistant to apoptosis because they express high levels of anti-apoptotic proteins. Prostaglandins and heat shock proteins (HSP) are cell-protective molecules and we thus investigated the expression of constitutive and inducible prostaglandin-synthases (COX-1 and COX-2, respectively) and of HSP70 in Ehrlich ascites tumor(EAT). Inducible nitric oxide synthase (iNOS) was also investigated. Seven to nine days after inoculation of $1x10^3$ tumor cells, EAT cells were harvested from the peritoneal cavity of BALB/c mice. $8X10^6$ cells were lysed and cell lysates were analyzed by immunoblotting using antibodies to COX-1, COX-2, iNOS and HSP70 and horseradish peroxidase-conjugated secondary antibody. After 7 and 10 days of tumor growth, aliquots of the ascitic fluid were analyzed by Flow Cytometry and trypan blue vital dye to quantify the apoptotic cells. EAT cells express considerable levels of HSP70 and COX-1, but neither iNOS nor COX-2 was detected. The number of apoptotic cells was $0.9x10^5(\pm 0.2x10^5)$ and $3.4x10^5(\pm 1.8x10^5)$ at days 7 and 10 respectively, corresponding to 1 and 1.4% of the cells in the ascitic fluid. The present study showed that the concentration of apoptotic cells is low during EAT growth and that the EAT cells are able to produce two groups of cell protective molecules, heat shock proteins and prostaglandins. This may explain the unusual capacity of Ehrlich tumor cells to establish and proliferate.

Key words: Ehrlich tumor, apoptosis, COX-1, HSP70