## NPP3 IS THE DOMINANT NUCLEOTIDE PYROPHOSPHATASE/PHOSPHODIESTERASE FAMILY MEMBER IN WALKER TUMOR CELLS

Buffon A.<sup>1</sup>, Wink M.R.<sup>2</sup>, Ribeiro B.V.<sup>1</sup>, Vieira, G.A.<sup>3</sup>, <u>Casali, E.A</u>.<sup>1,3</sup>, Zerbini L.F.<sup>4</sup>, Robson C.S.<sup>5</sup>, Sarkis J.J.F<sup>1</sup>

<sup>1</sup>Depto de Bioquímica and <sup>2</sup>Depto de Biofísica, UFRGS and <sup>3</sup>CUMIPA, Porto Alegre, RS, Brazil, <sup>4</sup>Joint Institute and <sup>5</sup>Harvard Medical School, Boston, Massachusetts, USA.

E-NPPs can hydrolyse 5'-phosphodiester bonds in nucleotides. Cells can coexpress enzymes for nucleotide hydrolysis with different catalytic properties and performing distinct physiological functions. ATP is cytotoxic in several tumour cell systems while adenosine presents a tumour-promoting activity. We described an e-NPP activity in Walker 256 tumor cell suspension. W256 is maintained through intraperitoneal passages in rats. W256 cell suspensions 98% viable were obtained from the ascitic fluid of a donor rat. Using p-Nph-5'-TMP as substrate, we demonstrate the major biochemical properties described as time incubation, protein concentration relation, divalent cation dependence and activity blockade by metal ion chelator. Total RNA was isolated from W256 cells and the cDNA was analyzed by PCR with primers for NPPs 1-3. The expression of NPPs was quantitatively analyzed by real-time PCR. W256 tumour cell suspension expresses principally an e-NPP3. We hypothesize that an E-NPP is co-localized with an e-NTPD and an e-5'-nucleotidase in W256 tumour cell suspension, as part of a multiple system for nucleotide hydrolysis. The e-NPP described here represents a novel insight into the control purinergic signalling in tumour cells. These results can generate news approach for protection mechanism against the tumoral process in circulation. Supported by: CNPq.