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Annexin A1 regulates apoptosis and phagocytic removal by macrophages of human malignant glioblastoma cells

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Annexin A1 (ANXA1) is an endogenous protein of the annexin superfamily with well-described anti-inflammatory properties in the peripheral system. It has also been detected in the central nervous system being constitutively expressed in brain glia cells, but its function is still unclear (*McArthur et al, 2010*). ANXA1 has been shown to be involved in apoptosis and differentiation of several cell lines. More recently, a new role for ANXA1 in phagocytosis and clearance of apoptotic cells has emerged. Moreover, apoptotic cells release ANXA1 and related peptides which stimulate phagocytosis of apoptotic neutrophils by macrophages (*Scannell et al, 2007*). Glioblastoma multiforme (GBM) is the most common malignant and resistant tumor of the central nervous system in humans and new therapeutic strategies are urgently required (*Terzis et al, 2006*). We have shown that the potential chemotherapeutic polyphenol xanthohumol (XH), isolated from Humulus Lupulus (*Zanoli & Zavatti, 2008*), induces apoptosis of human T98G glioblastoma cells by increasing reactive oxygen species and activating MAPK pathways (*Festa* et al, 2011). Then we have found, by western blotting and microscopic analysis, that XH (20 μM) up-regulates cytosolic levels of ANXA1 and induces translocation of the protein on the cell membrane of T98G cells in a time-dependent manner with significant effects observed after 24 h ($P<0.001$, n=5). On the basis of the above evidence, the aim of this work was to investigate the role of intracellular and cell membrane localized ANXA1 in glioblastoma T98G cells (obtained from ATCC stock). RT-PCR analysis has shown that XH (20 μM) up-regulates mRNA levels of ANXA1 after 16 h treatment ($P<0.01$ n=3). To demonstrate the involvement of ANXA1 in apoptosis of GBM cells we down-regulated ANXA1 expression with small interfering RNA (siRNA, 200 μM) using lipofectamine as transfection reagent and then apoptosis was analysed in the presence and absence of apoptotic stimuli. Importantly, apoptosis induced by XH was significantly reduced in siRNA-ANXA1 transfected cells where western blot analysis shows a significant down-regulation of ANXA1 protein levels ($P<0.001$, n=5). To investigate the role of ANXA1 expression on the cell membrane of T98G cells as potential “eat-me” signal we studied phagocytosis of apoptotic cells by human macrophages. We incubated apoptotic T98G cells with human blood monocyte derived macrophages (M□) obtained from healthy donors. After co-incubation period we analysed the percentage of M□ phagocytosing the apoptotic cells by cytofluorimetric FACS analysis and by confocal microscopy. Our results show that XH (20 μM) induces phagocytosis of apoptotic T98G cells by human M□ in a concentration-effect manner ($P<0.01$, n=3), a processes that is dependent on caspase mediated apoptosis. ANXA1 acts as an “eat-me” signal on the cell membrane of T98G cells, and interestingly, apoptotic siRNA-ANXA1 transfected cells are not completely ingested by M□. ANXA1 was also detected by western blotting in supernatants of apoptotic cells; the incubation of enriched supernatants from apoptotic cells enhanced the percentage of phagocytosis by M□ ($P<0.05$, n=3). These results demonstrated that ANXA1 is involved both in the apoptosis and phagocytosis of glioblastoma cells. This study shows a possible role of ANXA1 in maintenance of brain homeostasis and may lead to novel therapeutic approaches for neuro-inflammatory diseases and chemotherapy targets in the treatment of glioblastoma multiforme.

References

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