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Research paper

The study of the calpain and caspase-1 expression in ultrastructural dynamics of Ehrlich ascites carcinoma necrosis

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ABSTRACT

An expression of calpain and caspase-1 as well as the concomitant ultrastructural alterations were investigated during necrosis of the mouse Ehrlich ascites carcinoma. The calpain expression was registered at 0 h and 1 h although caspase-1 did not induce any signals during these time periods. The rise of the cytoplasmic lytic zones contacted by calpain antibodies was identified as a morphologic event corresponding to the expression of calpain. Lytic zone's distribution followed by the appearance of the calpain/caspase-1 clusters assigned for lysis of the Golgi vesicles and ER. Also, the microapocrine secretion of the vesicles containing the calpain/caspase-1 clusters was detected. Further, the lysis of the plasma membrane occurred due to progression of intracellular lysis. Rupture of the plasma membrane resulted in the termination of secretion and dissemination of cell contents. The nuclei still had their normal shape. Nuclear lysis continued to rise with intranuclear lytic zones, of which the progression was accompanied with the presence of calpain/caspase-1 clusters. The data contribute to the concept of the initial role of calpain for tumor cell destruction, provide first evidence of the calpain/caspase-1 pathway in tumor cells, and highlight microapocrine secretion as a possible tumor cell death signalling mechanism.

1. Introduction

Tumor resistance to apoptosis is an essential problem of anticancer therapy. Accordingly, inducing a necrosis as an alternative cell death form is an attractive approach. It is suggested that tumors might be sensitive for necrosis stimulation. Besides, necrotic debris might become a reason for the anticancer immune response resulting in treatment improvement (Moriwaki and Chan, 2013; Cho and Park, 2014).

Understanding of necrotic machinery could make it possible to adopt a better strategy for a tumor necrotic collapse (Moriwaki and Chan, 2013; Cho and Park, 2014). However, this task is not easy taking into account the heteromorphy of necrosis types. Necrosis was initially characterised as genetically independent cell death induced by extreme physical or chemical stress. However, the regulated necrosis-like forms – necroptosis, secondary (post-apoptotic) necrosis, pyroptosis and oncosis were revealed (Mills et al., 2002; Fink and Cookson, 2005; Vanden Berghe et al., 2010; Moriwaki and Chan, 2013; Celardo et al., 2013; Sollberger et al., 2014).

Interestingly, despite that molecular cascades are different in various necrosis types, the morphologic similarity is their feature. For both the non-tumor and tumor cells the necrosis was featured with damaged lytic organelles, nuclear disintegration, and a broken plasma membrane causing the release of the inflammatory cellular content into the extracellular space (Moriwaki and Chan, 2013; Vanden Berghe et al., 2010; Cho and Park, 2014; Osorio-Vega et al., 2016). Cell lysis is a common attribute of all necrosis forms. Therefore, the term “lytic cell death” (Berwin et al., 2001) seems appropriate to characterise the general necrosis concept.

It was noted by Festjens et al. (2006) that it is still impossible to clearly discriminate between the initiation, propagation and execution phases of necrotic cell death. The triggering alterations that could be upstream for necrosis are still evasive. However, the initial changes should be found to choose the optimal stimuli for induction of pronecrotic activity (Vanlangenakker et al., 2012). In this regard, more detailed insight into the necrosis of tumor cells is very necessary.

Necrosis has been reported as caspase-independent cell death

Abbreviations: cy, cytoplasm; n, nucleus; mv, microvillus; pm, plasma membrane

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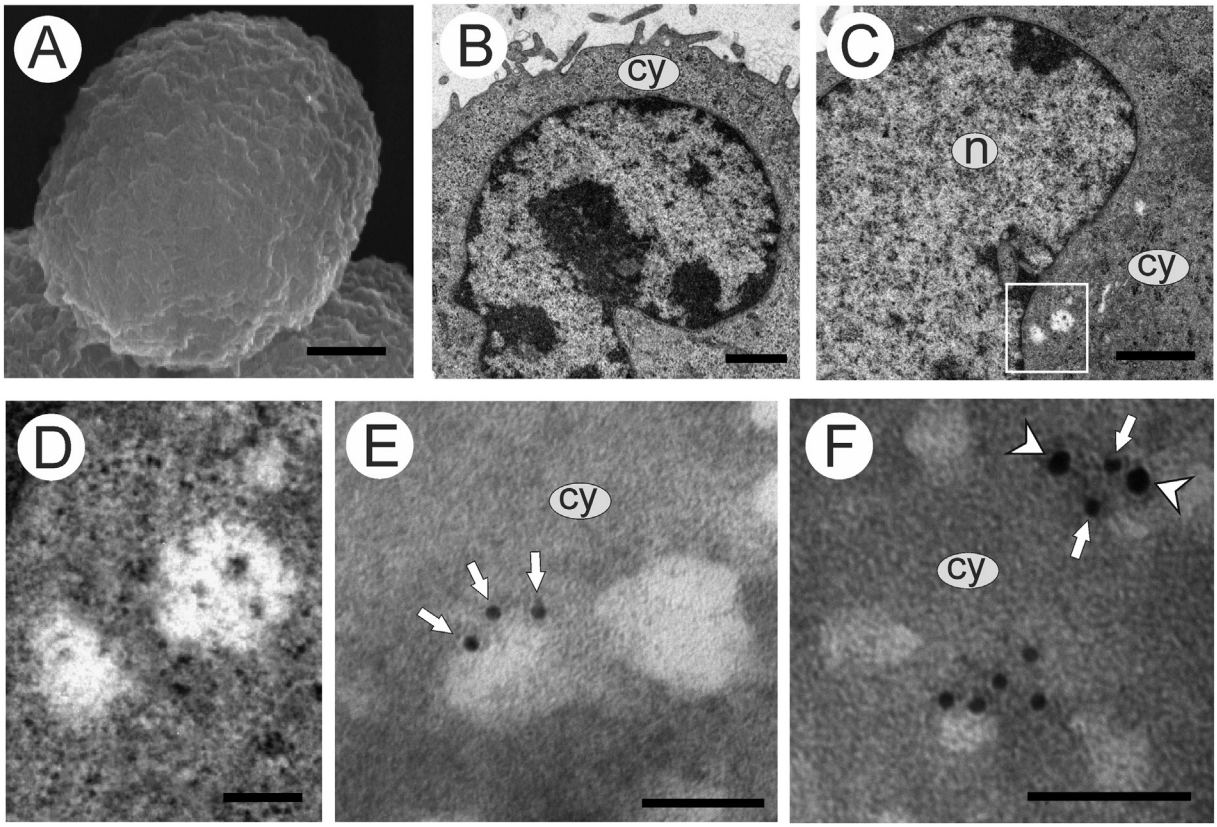

Fig. 1. Cells of cultured mouse Ehrlich ascites carcinoma (EAC) during early necrosis induced by cucumarioside A_2–2.
Cell surface typical for 0, 1, 2 and 3 h time periods acquired by scanning electron microscopy (SEM); note absence of any surface damage (A).
0 h stage; cell of EAC by transmission electron microscopy (TEM); note absence of any alteration in the cytoplasm (B).
1 h stage; cells of EAC by TEM; note light zones in the cytoplasm (shown by square) (C).
Enlarged image of the light zones shown by square in the previous image (D).
1 h stage; cells of EAC by immunoelectron microscopy (iEM); note 12 nm gold particles (shown by arrows and mean calpain) located by the light zone (E).
2 h stage; cells of EAC by iEM; note cluster consisting of 12 nm gold particles (calpain) (at the bottom of the image) and cluster consisting of both the 12 nm gold particles (arrows) as well as 18 nm gold particles (arrowheads) corresponding to calpain and caspase-1 respectively (F).
Cy – cytoplasm, n – nucleus.
Scale bars = 1 μm (A), 0.5 μm (B, C, F), 0.1 μm (D, E).

pattern (Festjens et al., 2006). However, it was found that such a necrosis-like cell death type as pyroptosis is mediated by caspase-1. This phenomenon was discovered in macrophages infected with *Salmonella* and *Shigella* species (Fink and Cookson, 2005; Bergsbaken et al., 2009; Sollberger 2014). It seems constitutive to check if this protease activity is not restricted by lytic cell death in macrophages. It is especially important to figure out if caspase-1 is involved in necrosis of the tumor cells.

According to some reports, in tumor cells the expression of caspases is anticipated by the expression of calpain. An inductive role of calpain has been shown for caspase-3 in Burkitt's lymphoma cells (Waterhouse et al., 1998), for caspases-9, -3 and -7 in large cell lung carcinoma (Gil-Parrado et al., 2002), and for the caspase-3/7 pathway in melanoma cells (Del Bello et al., 2007). It seems necessary to check if the expression of caspase-1 could be also anticipated by the expression of a calpain.

Mouse Ehrlich ascites carcinoma (EAC) is a popular model for the study of various aspects of tumor destruction due to its high sensitivity to therapeutic treatment (Bhattacharyya et al., 2003; Vieira et al., 2010; Ozaslan et al., 2011; Da Mota et al., 2012; Osman et al., 2015). It was previously shown that apoptosis in EAC could be completed by secondary or apoptotic necrosis, and more switch cases from apoptosis to necrosis were registered with the application of higher concentrations of cucumarioside A_2-2 (Menchinskaya et al., 2013; Reunov et al., 2015). Thus, the EAC treated with cucumarioside A_2-2 is a proper model to figure out if calpain/caspase-1 pathway is expressed in these tumor cells.

This study was undertaken to enlighten the poorly understood scenario of EAC cells necrosis. The focus was centered on finding cell alteration that is upstream regarding to this process. A calpain and caspase-1 expression was investigated in connection to necrosis consequent steps.

2. Materials and methods

2.1. Animals

CD-1 mice weighing 18–20 g were purchased from RAMS nursery (Russia) and kept at the animal facility in standard conditions. All experiments were conducted in compliance with all of the rules and international recommendations of the European Convention for the Protection of Vertebrate Animals Used for Experimental Studies.

2.2. Ehrlich carcinoma cell isolation

EAC cells were inoculated into the peritoneal cavity of mice of both sexes. For the experiments, tumor cells were collected from the ascetic fluid 7–10 days after tumor inoculation. The mice were killed by perivisceral dislocation, and the ascetic fluid containing tumor cells was collected with a syringe. The plates with ascetic fluid were incubated in a CO_2 incubator. To remove exudates, the cells were washed in PBS twice by centrifugation at 1500 rpm (450*g*) for 10 min using a Heraus