CYTOARCHITECTURAL CHANGES DURING RETINOIC ACID-INDUCED APOPTOSIS IN F9 EMBRYONAL CARCINOMA CELLS

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The F9 murine embryonal carcinoma (EC) cell line has been widely used as a model for the study of retinoic acid-induced differentiation (Alonso A. et al 1991). These F9 cells differentiate under exposure to retinoic acid (RA) into the three types of extra-embryonic endoderm (primitive, visceral and parietal), depending on the culture conditions and RA concentration used. More recently, we have reported that RA can trigger an apoptotic response, or programmed cell death, associated to the early stages of differentiation in F9 EC cells (Atencia et al. 1994).

This apoptotic effect of retinoic acid is observed when cultures of undifferentiated F9 cells are treated with physiological doses of RA (1mM) for 48 hours, and reaches their maximal expression after 72 h. of treatment, affecting over 30 % of cells.

Figure 1. A: undifferentiated cell. B: differentiated cells C: apoptotic cell.

By using this experimental system, we have made an approach to the dynamics of the apoptotic process through the analysis of the morphological changes that affect to the inner membranes and several cytoskeletal components of the apoptotic F9 cells. Ultrastructural study (Fig. 1) showed that treated cultures were formed by three cells types. A) Undifferentiated F9 cells. B) Differentiated cell -polarized cuboid cells joined by well-defined tight junctions and microvilli covered the outer cell surface-,C) Apoptotic cells.

During apoptosis the disappearance of internal membranes are progressive (Fig. 2). In the first phase the endoplasmic reticulum dilates and the cisternae forming vesicles that fuse with the cell membrane, voiding their contents extracellularly (A). In the second phase the cromatin becomes highly condensed into spherical domains that ultimately extrudes through the nuclear envelope, forming apoptotic bodies. The pore complexes appear clustered in the nuclear membrane (B) -to form a mirrored image- in areas corresponding to detachment of apoptotic bodies (C). In the third phase the nuclear envelope is scattered in small (D) and/or long (E) structures that are placed near/or fused with cellular membrane (F).

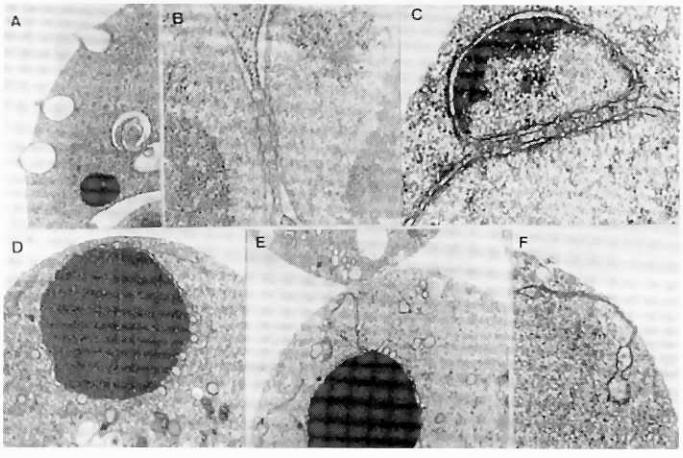

Figure 2. Evolutive process of internal membranes during apoptosis

We have also analysed by confocal microscopy the location of the actin microfilaments and several cytoskeletal proteins such β-tubulin, vimentin and the embryonic cytokeratin EndoA, in F9 embryonal carcinoma cells undergoing apoptosis during the stages previous to their fragmentation. Our results show that the actin localizes in the centre of the disrupting cell forming a three-dimensional structure which suggests the existence of a fully functional contractile system involved in the fragmentation of the cell and the formation of apoptotic bodies (Figure 3). The position of this molecule in the apoptotic, pre-fragmented F9 cells could also be explained as a collapse of all the cytoskeletal framework, but its spatial organisation, defining the blebbing zones and showing sporadic, ring-like structures resembling those that are formed during the mitotic process, appears to be the consequence of a driven process rather than a random one. However, we have observed that this organisation seems to be specific for the microfilament

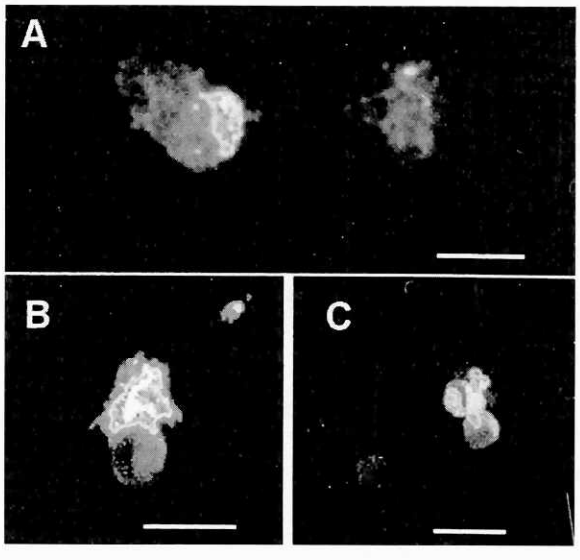

Figure 3. Actin organisation in F9 cells. Extended focus images from A) a normal mitotic cell. B) and C) apoptotic cells (bar=3μm)

network since the analysis of other cytoskeletal components such as the intermediate filaments EndoA and vimentin (characteristically expressed by the differentiating F9 cells) shows that these molecules appear randomly distributed or are simply absent in the apoptotic cells (Figure 4). Moreover, the expression of vimentin starts only after 96 h. of exposure to RA 24 h. later than the highest apoptotic activity observed in this experimental system. This fact excludes the vimentin from any role in the formation of apoptotic bodies, at least in the F9 cell system. Similarly, the expression of the cytokeratin EndoA, a differentiation marker that is expressed by the extraembryonic endoderm of the normal blastocyst (Oshima, R.G., 1981) and the endodermal derivatives obtained from the RA treated F9 EC cells, seems not to be involved in the apoptotic machinery, although its expression can be observed, in normal, non-apoptotic cells, after 48 h. of RA treatment.

The microtubule studies on undifferentiated cells showed a similar arrangement than any other cells in culture. Regarding to apoptotic cells, we have not detected any sign leading to suggest an active role of the microtubules in this process by means of immunofluorescence assays. Moreover, the β-Tubulin expression of the apoptotic cells appears to be scattered and depolymerised, without displaying well-defined cell structures (Figure 5).

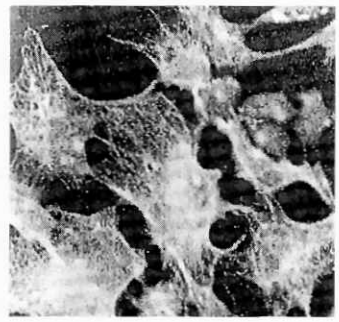

Figure 4. EndoA cytokeratin expression on differentiated cells. (bar=10 μm)

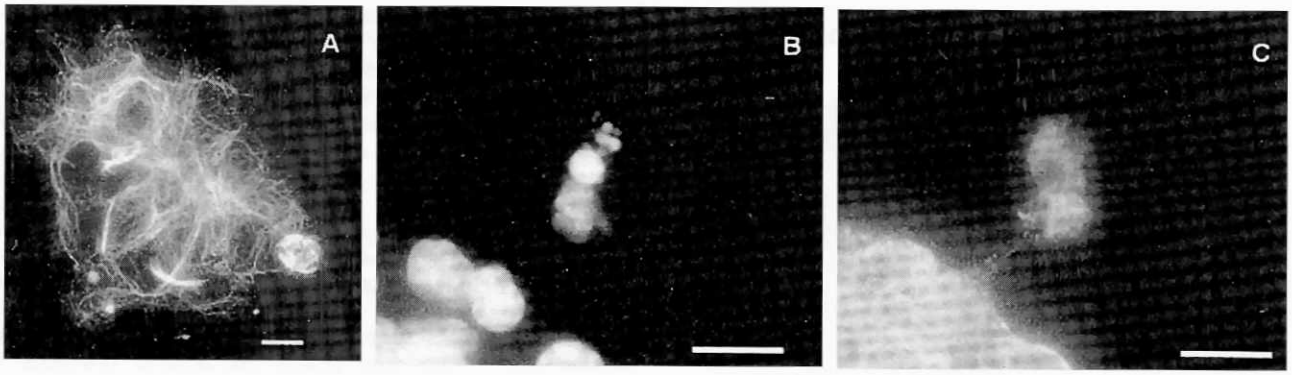

Figure 5. Tubulin expression on differenciated (A) and apoptotic cells (C). B and C correspond to a double-stain immunofluorescence assay, displaying nuclei with DAPI (B) and tubulin arrangement with FITC (C). Bar=10μm.

In summary, our results suggest that as in gene activation mechanisms, there would be a progressive actuation of certain cell process that eliminates those cells that are not ready to assume the essential changes leading from an undifferentiated, highly proliferative state to a more complex, differentiated phenotype.

Acknowledgements

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