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Reiner U. Jänicke

► **To cite this version:**

Reiner U. Jänicke. MCF-7 breast carcinoma cells do not express caspase-3. *Breast Cancer Research and Treatment*, 2008, 117 (1), pp.219-221. 10.1007/s10549-008-0217-9 . hal-00486600

HAL Id: hal-00486600

<https://hal.science/hal-00486600>

Submitted on 26 May 2010

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MCF-7 breast carcinoma cells do not express caspase-3

Reiner U. Jänicke

Published online: 14 October 2008
© Springer Science+Business Media, LLC. 2008

Apoptosis, a fundamental process essential for normal tissue homeostasis and development, is closely associated with the activation of a class of aspartate-specific cysteine-dependent proteases, called caspases, that lead to the demise of the cell via limited proteolysis of a multitude of cellular substrates [1, 2]. Caspases are expressed as inactive zymogens that become activated upon cleavage by other caspases in a so-called caspase activation cascade, or by mere oligomerization instigated by the formation of large multi-protein complexes such as the death-inducing signaling complex (DISC) or the apoptosome [3, 4]. Whereas DISC formation occurs via the so-called extrinsic death pathway that is instigated by activation of members of the death receptor family such as CD95, tumor necrosis factor receptor (TNF-R) or the receptors of the TNF-R-related apoptosis-inducing ligand (TRAIL), the apoptosome is formed following activation of mitochondria and is hence termed the intrinsic or mitochondrial death pathway. Based on their order in cell death pathways, caspases can be divided into initiator (caspase-2, -8, -9, and -10) and effector (caspase-3, -6, and -7) caspases. Among them, caspase-3, a member of the latter group, is absolutely crucial for apoptosis induction, as this enzyme is not only activated downstream of both the extrinsic and intrinsic death pathway, it is also responsible for the cleavage of the majority of substrates known so far [1, 5]. More importantly, with the proteolysis of discrete substrates, caspase-3 evokes some of the typical morphological and biochemical alterations associated with apoptosis. For instance, whereas

the caspase-3-mediated cleavages of α -fodrin, gelsolin, rho-associated kinase-1 (ROCK-1) and p21-activated kinase 2 (PAK2) contribute to membrane blebbing, cleavage of the inhibitor of the caspase-activated DNase (ICAD) leads to the typical DNA fragmentation pattern observed in apoptosis [1]. Furthermore, with the cleavage-mediated activation of the calcium-independent phospholipase A2 and subsequent production of the chemotactic phospholipid lysophosphatidylcholine, caspase-3 appears to be also responsible for the generation of so-called “eat-me” signals that induce migration of phagocytes to the site of apoptotic cell death [6]. Thus, caspase-3 not only instigates and pursues the demise of a cell, but, in addition, makes sure that the corpse is properly disposed, a function crucial for avoiding inflammatory processes.

Hence, determination of the processing and activation of caspase-3 are common means to assess apoptotic signal transduction pathways in numerous cell lines of varying origin. Curiously, several reports still claim the presence of caspase-3 in the breast carcinoma cell line MCF-7 in which this enzyme is supposed to contribute to apoptosis signaling [7–15]. This was not only demonstrated indirectly via fluorometric assay systems measuring caspase-3-like activities—that might be also elicited by the closely related caspase-7—but by Western blotting analyses demonstrating directly the presence of this protease in MCF-7 cells. However, in addition to the lack of caspase-10 [16], an initiator caspase in the extrinsic death pathway, MCF-7 cells do also not express caspase-3 [17]. Ten years ago, we demonstrated unambiguously that the lack of caspase-3 in these cells is caused by a 47-base pair deletion within exon 3 of the *CASP-3* gene resulting in the skipping of this exon during pre-mRNA splicing and introduction of a premature stop codon at position 42 that completely abrogates translation of the *CASP-3* mRNA [17]. Although caspase-3-deficient MCF-7

R. U. Jänicke (✉)
Laboratory of Molecular Radiooncology, Clinic and Policlinic
for Radiation Therapy and Radiooncology, University
of Düsseldorf, Universitätsstrasse 1, 40225 Düsseldorf, Germany
e-mail: Janicke@uni-duesseldorf.de

cells are still sensitive to cell death induction by several stimuli including TNF, staurosporine [17, 18] and various DNA damaging agents [19, 20], death of these cells occurs in the absence of DNA fragmentation. In addition, the distinct morphological features typical of apoptotic cells such as shrinkage and blebbing are also not evident in caspase-3-deficient MCF-7 cells. As introduction of caspase-3 restored all of these events, our results—and also those of many other laboratories—clearly showed that this protease is crucial for these typical biochemical and morphological changes of cells undergoing apoptosis.

Although caspase-7 might compensate under certain circumstances for the lack of caspase-3, it is well appreciated that the executioner caspases 3, 6 and 7 perform distinct, non-redundant roles during the demolition phase of apoptosis [21]. In addition, it was shown that caspase-3 is the primary activator of apoptotic (low molecular) DNA fragmentation via cleavage-mediated inhibition of ICAD [22]. Thus, it is puzzling that caspase-3-deficient MCF-7 cells exhibit DNA fragmentation and other apoptotic characteristics following cell death induction as described [7–15]. While it is still possible that caspase-3-deficient MCF-7 cells display high molecular weight DNA fragmentation that might be occasionally mistaken for the typical apoptotic DNA fragmentation, the detection of a caspase-3 protein in MCF-7 cells [7, 8, 11, 13–15] with a partially deleted *CASP-3* gene is cause for concern. Such discrepant findings may be partially explained by the use of inappropriate antibodies that cross-react with other caspase-3-unrelated proteins in cellular extracts, a phenomenon we have also observed previously (unpublished data). Alternatively, as there are several variants of MCF-7 cells around, the examined cultures might not contain the original MCF-7 cell line, and a karyotyp analysis to clarify this issue would be appropriate. Nevertheless, similar to our recent note of caution with regard to the presence of caspase-10 in the mouse [23], this letter should make a general readership aware of the fact that the original MCF-7 breast cancer line that can be obtained from the American Tissue Type Culture Collection (ATCC) does not express the caspase-3 protein. Hopefully, this misconception can be avoided in the future.

Acknowledgment This work was supported by the Collaborative Research Center “Extrinsic Ageing” funded by the Deutsche Forschungsgemeinschaft (SFB 728/B1).

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