Differentiation between apoptotic and non-apoptotic cell death using diffusion-weighted MR

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Purpose

Cells that are dying via apoptotic or non-apoptotic cell death pathways exhibit characteristic changes in size, nuclear architecture, and membrane integrity [1, 2]. We hypothesised, based on basic diffusion modeling, that these morphological changes affect the diffusion properties of cell cultures. In the course of this study we also established some fundamental relationships between the ADC and cell culture metrics.

Methods

The ADC values were determined for cell pellets by using 2 b-values (0 and 1000 s/mm^2) on a 1.5-T MR imaging system (Philips Medical Systems, Best, The Netherlands). Perfusion-related incoherent microcirculation is absent in vitro, thus the ADC can be directly calculated from: ADC = $\ln(S_0/S_{1000})/1000$. The physical properties of cells (HeLa S3, HL-60 and CV-1) that were suspended and centrifugated (200–3200 *g*) were measured under a microscope (Zeiss, Axioscop) at a magnification of ×40.

Results and Discussion

We first established that the ADC of HeLa S3 cell pellet is strongly correlated with the cell area (CA; ADC = -1.552 × CA + 1.6864, R^2 = 0.92918) (Fig. 1A). CA includes the nuclear and cytoplasmic area, as measured with AutoMeasure (v4.5, Zeiss). The results are consistent with the notion that increased intracellular water fraction decreases ADC. However, the same expression as for HeLa S3 did not describe correctly the relation between ADC and CA of the other cell lines (Fig. 1A). However, we did find another physical cell parameter that allowed us to calculate the ADC independent of the cell types tested in this study. Indeed, the cell perimeter length (CPL) shows a linear and very strong correlation with the ADC for all cells (ADC = -1.3647 × CPL + 1.1001, R^2 = 0.97827) (Fig. 1B). Thus, the total plasma membrane area, quantified through the CPL, is a very strong determinant of the cell culture's ADC.

Finally, we tested the hypothesis that we could understand the changes in ADC during cell death using these insights. When cells were induced to undergo apoptosis by camptothecin or staurosporine, the ADC of these cell cultures gradually declined (Fig. 2). This finding was consistent with an intact but blebbing plasma membrane and nuclear fragmentation, characteristics of apoptosis. The blebbing of the membrane results in both an increased CPL and an increased CA. In contrast, the ADC values increased in the cells that were induced to non-apoptotic death by tumor necrosis factor α or a caspase inhibitor (Z-VAD-fmk) (Fig. 3). These findings were consistent with a ruptured plasma membrane. A membrane rupture results in decreased CPL.

Conclusion

These results suggest that the area and integrity of the plasma membranes, as quantified by the total CPL, are critical determinants of the diffusion characteristics on the cellular level. The evolution over time of the ADC values is characteristic of whether the cells die via apoptotic or non-apoptotic (necrotic) pathway. Therefore, this can be used as a cancer biomarker for differentiating between different pathways of cell death, e.g. in testing the effects of cancer drugs in vitro.

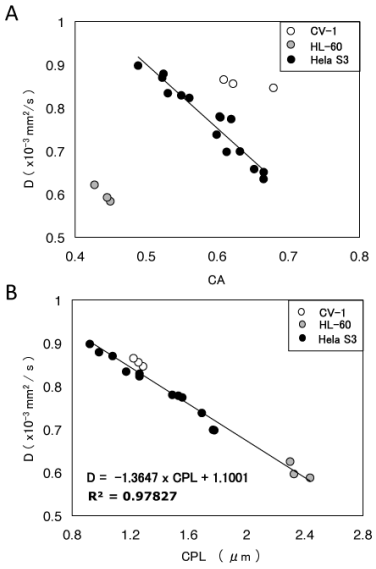

Fig. 1.—Correlation between D values and cell metrics
A, Graph shows a linear correlation between D values and cell area (CA) of HeLa S3 cells (D = -1.552 × CA + 1.6864, R^2 = 0.92918). Plots of D values relative to cell density ratios of CV-1 and HL-60 cells are superimposed on the graph for HeLa S3 cells. Note that plots for CV-1 and HL-60 cells greatly deviate from the linear correlation between D values and cell density ratio for HeLa S3 cells.
B, Graph shows a linear correlation between D values and cell perimeter length (CPL) per pellet area (CPL; mm/mm^2) of HeLa S3, HL-60, and CV-1 cells (D = -1.3647 × CPL + 1.1001, R^2 = 0.97827). CA and CPL were determined using dedicated software (AutoMeasure, ver. 4.5, Zeiss).

References

1. Leist M and Jäättelä M. Nat Rev Mol Cell Biol 2001;2:1-10.
2. Golstein P and Kroemer G. Trends Biochem Sci 2006;32:37-43.

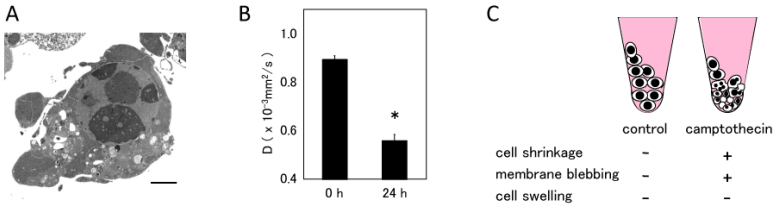

Fig. 2.--Molecular diffusion of camptothecin-treated (apoptotic) HeLa S3 cells
A, Transmission electron microscopy shows chromatin condensation, and blebbing of the intact plasma membrane of camptothecin -treated (24 h) HeLa S3 cell. Scale bar = 2 μm.
B, Graph shows time-dependent decreases in D values of camptothecin-treated (8 h and 24 h) HeLa S3 cells. All cells that adhere to or detach from the bottom of culture dishes were collected, resuspended in PBS, centrifugated, and assessed for D values. The data are means ± s.d. from 3 independent experiments. *, p <0.001.
C, Schematic representation and cellular characteristics of cell pellets containing camptothecin-treated(24 h, apoptotic) HeLa cells.

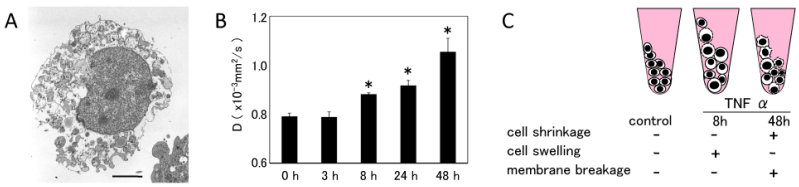

Fig. 3.--Molecular diffusion of TNFα-treated (necroptotic) L929 cells
A, Transmission electron microscopy shows disruption of plasma membrane and swelling of mitochondria of TNFα-treated(48 h) L929 cell. Scale bar = 2 μm.
B, Graph shows a gradual increase in D value of necroptotic L929 cells. *, p <0.001.
C, Schematic representation and cellular characteristics of cell pellets containing TNFα-treated (necroptotic, 8 h and 48 h) L929 cells.