Letter to the Editor

Reply to 'Role of glucose-6-phosphate dehydrogenase for oxidative stress and apoptosis'

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Dear Editor,

In the letter 'Role of glucose-6-phosphate dehydrogenase for oxidative stress and apoptosis', Efferth *et al.*¹ summarised reports favouring the role of G6PD in protection from redox-stress-induced apoptosis. Therein, the authors also referred to our studies described in *Cell Death and Differentiation*.² Efferth *et al.*¹ compared our study with the one they published in 1995³ describing the induction of apoptosis in peripheral mononuclear cells (PMBC) carrying a G6PD mutation (G6PD Aachen). In our paper,² we investigated in wild-type and G6pd-deleted (*G6pdΔ*) mouse embryonic stem (ES) cells the signalling events activating an apoptotic pathway downstream GSH oxidation. These authors objected against the statement 'for the first time a role for G6PD in the protection from redox-imbalance-induced apoptosis and necrosis has been clearly assigned',² claiming that such a finding was reported in their 1995 paper.³

In our opinion, the two studies are not comparable. Efferth *et al.*³ used PMBC carrying the G6PD mutation Aachen, but Kahn *et al.*⁴ had already described the main characteristics of this variant and, in particular, the severe enzyme deficiency in erythrocytes, contrasting with an almost normal activity observed in white blood cells. Efferth *et al.*¹ also state: 'Pandolfi *et al.*⁵ reported G6PD is essential for defence against oxidative stress using knockout mice', but Pandolfi *et al.*⁵ never used knockout mice, their study carried out on mouse ES cells described to be G6PD null. In addition, in a successive paper, the same authors⁶ published that their ES cell lines retain a small amount of G6PD activity: thus, these ES cell lines appear to be severely G6PD deficient rather than G6PD null. In our paper,² the study was carried out, for the first time, on ES *G6pdΔ*, which are completely unable to produce both G6pd transcript and G6PD.⁷

Moreover, Efferth *et al.*^{3,8} analyse the induction of apoptosis by various agents including cytostatic drugs (daunorubicin), gamma-irradiation, glucocorticoids (dexamethasone) and UV irradiation. All these factors are not oxidative agents *per se*, but they can induce different kinds of damages in the cells (i.e. inhibition of enzymes, DNA and membrane damages), ultimately leading to generate reactive oxygen species (ROS). Under these conditions, it is difficult to ascertain whether apoptosis is induced by ROS, or is the result of damages produced by the synergistic effects of these agents in G6PD-deficient cells.

In addition, after the apoptotic insult, Efferth *et al.*^{3,8} did not test the levels of GSH and NADPH and of their oxidised forms, nor measured the production of ROS and did not even analyse

the protective role against apoptosis of antioxidant such as GSH or N-Acetyl-Cysteine (NAC).

In contrast, in our experiments published in *Cell Death and Differentiation*,² we used diamide (a sulfidryl group oxidizing agent), menadione (a quinone compound that induces the production of superoxide radicals) and H₂O₂, substances that directly induce oxidative stress on the cells. We also reported that the induced apoptotic effect on ES *G6pdΔ* cells was reverted by reintroduction of the G6pd gene or by the presence of GSH or NAC² (Figure 1). In the same paper, we reported an increased production of ROS in presence of diamide in *G6pdΔ* ES cells compared to wild-type ES. We had previously analysed the cellular redox state in the presence of diamide and observed that NADPH and GSH in ES *G6pdΔ* cells dramatically shift to their oxidised forms.⁷ In contrast, under the same conditions, wild-type ES cells are able to rapidly and intensely increase the activity of the pentose phosphate pathway in response to the oxidant.⁷ Moreover, we reported that ES *G6pdΔ* cells show an increased sensitivity to apoptosis only if treated with oxidative agents, whereas in the presence of other apoptotic-inducing agents including UV

Figure 1 Treatment with the antioxidants protects *G6pdΔ* ES cells from cell death. Cell death in *G6pdΔ* cells was evaluated by counting blue cells after Trypan blue staining. *G6pdΔ* cells were treated with 150 μ M diamide (DIA), 1.5 mM H₂O₂ and 100 μ M menadione (MQ). N-Acetyl-L-cysteine (1 mM) or GSH (2.5 mM), added with oxidant agents, were tested for their ability to rescue *G6pdΔ* cells from cell death

irradiation, they resulted in being equally sensitive or even slightly more resistant than wild-type ES cells.²

For all these reasons, we still believe that our paper demonstrates for the first time a protective role of G6PD from redox-stress-induced apoptosis and necrosis.

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