very low in pre-treatment samples and it is strongly increased in 18 out of 22 patients (81.8%) after therapy (Supplementary Figure 3).

Altogether, our findings show that ΔN-p73 is a transcriptional target of the PML/RARα oncogene. This results in the transcriptional repression of ΔN-p73 providing one potential molecular basis underlying the lack of ΔN-p73 expression in a large subset of APL leukemias. The role of PML/RARα in ΔN-p73 repression is confirmed by the ability of RA to restore its expression both *in vitro* and *in vivo*. The observation that ΔN-p73 expression induces a number of differentiation markers in APL cells and cooperates with RA-induced differentiation *in vitro* suggests that ΔN-p73 might be necessary for proper myeloid differentiation. Indeed, ΔN-p73 expression is modulated during muscle and kidney differentiation.[8,9] Although ΔN-p73 has been mainly involved in the inhibition of p53-, TAp63- and TAp73-dependent transcription of target gene promoters containing p53REs, a series of recent evidences indicates that ΔN-p73 may directly and indirectly activate transcription from a number of target genes.[10] Thus, the ability of RA to remove the differentiation block of APL leukemias and to restore ΔN-p73 expression might result in the activation of a specific subset of yet unidentified genes involved in myeloid differentiation.

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Role of EndoG in development and cell injury

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Dear Editor,

Recent studies have presented contradictory evidence about the role of endonuclease G (EndoG) in early development and cell injury. While multiple data suggest that this enzyme is important in embryogenesis[1] and injury-induced cell death (see Supplementary Table 1), two recent reports claim that EndoG is dispensable in both processes.[2,3]

EndoG is currently identified as one of the most active cell death endonucleases. It is a nuclear DNA-coded mitochondrial endonuclease that has a unique site selectivity, initially attacking poly(dG).poly(dC) sequences in double-stranded DNA.[4] EndoG's expression varies among the tissues.[5] The enzyme was first localized in the intermembrane space of mitochondria, while later found to be tightly attached to the inner membrane of mitochondria.[6] Mammalian EndoG is synthesized as an inactive 32 kDa propeptide. The mitochondrial signal peptide is cleaved off by an unknown proteinase upon entering the mitochondria and the mature active 27 kDa EndoG can be released from mitochondria during apoptosis. Even though the protein does not have a known nuclear localization signal, it moves to the nucleus, where it cleaves DNA apparently without sequence specificity. Unlike other cell death endonucleases, EndoG can be often seen in the nucleus at the moment of DNA fragmentation as measured by the TUNEL assay.[7] EndoG is highly cytotoxic. Overexpression of extramitochondrially active EndoG in HeLa and CV1 cells induced cell death by acting alone, while the expression

of an inactive mutant of EndoG did not induce cell death.[8] The compartmentalization of mitochondria plays the major role in EndoG trafficking. The apoptosis induced by EndoG is caspase independent.[6,9] The enzyme was shown to co-operate with exonuclease and another cell death endonuclease, deoxyribonuclease I (DNase I).[10]

To explain DNA fragmentation observed in the early studies of apoptosis, Willie[11] suggested that this type of cell death is associated with 'activation of an intracellular, but non-lysosomal, endonuclease'. Later, several such enzymes became known. It also became obvious that apoptosis is not only associated with but is also induced by some cell death endonucleases. The mode of EndoG action is very similar to the one of DNase I and other cell death endonucleases. A significant amount of active ready-to-use or easily released enzyme is accumulated outside the nucleus or at least apart from nuclear DNA. If the compartmentalization fails, for example due to damage of the nuclear envelope, the endonuclease moves to the nucleus and induces the fragmentation of DNA (Supplementary Figure 1).

Studies on *Caenorhabditis elegans* first suggested that EndoG may be important for development. The reduction of cps-6, a homolog of EndoG, by using small interfering RNA (siRNA) or genetic mutation affected normal DNA degradation and caused the delayed appearance of cell corpses during development in *C. elegans*.[12] Recent studies utilizing EndoG knockout (KO) mice resulted in the controversy regarding the role of EndoG. Initially, Zhang *et al.*[1] showed that homozygous EndoG$-/-$ KO mice are not viable, which supported the importance of this enzyme for apoptosis during early development. Later, Irvine *et al.*[3] and then David *et al.*[2] had independently developed viable EndoG$-/-$ mouse knockouts. All three reports used mice of different backgrounds, which perhaps may explain the contradiction. The two latest studies suggested that the viability of EndoG$-/-$ mice was provided by avoiding the inactivation of the *D2Wsu81e* gene that partially overlaps with the *EndoG* gene. Although the role of the adjacent gene is unknown, it is also probably a possibility. Both studies went further to determine whether EndoG is important for injury-induced apoptosis. Irvine *et al.*[3] isolated splenocytes from adult mice and subjected them to etoposide or actinomycin D treatments. In the study by David *et al.*,[2] fibroblasts were isolated from embryos and treated with a panel of apoptosis inducers. Both studies determined that primary EndoG$-/-$ cells are as sensitive to injury-induced apoptosis as EndoG$+/+$ cells, which was interpreted as the evidence for the absence of the role of EndoG in injury-related cell death. Surprisingly, in both studies, the level of EndoG expression in wild-type (WT) cells before the treatment was not assessed. This makes the interpretation of the results difficult.

On the other hand, there is a large body of evidence that the presence of EndoG is essential for injury-related cell death (see Supplementary Table 1). For example, Bahi *et al.*[13] used the lentivirus-delivered silencing of EndoG and showed that this endonuclease is important for the DNA fragmentation induced by ischemia in neonatal cardiomyocytes. The silencing of EndoG alone by RNA interference inhibited cell death induced by oxidative stress in human mesenchymal progenitor cells and rat primary hepatocytes. The EndoG location site may indicate that this enzyme is not an instrument of immediate response to cell injury, and that the release of EndoG from mitochondria is crucial. The role of oxidative stress in the induction, release from mitochondria and nuclear import of EndoG was confirmed by several studies. The overexpression of superoxide dismutase 1 (SOD1) in transgenic rats attenuated the nuclear import of EndoG and apoptosis after spinal cord injury.[14] In another study, wood smoke extract was shown to induce oxidative stress-mediated caspase-independent apoptosis in human lung endothelial cells that involves EndoG.[15] Mitochondrial DNA point mutations caused profound energetic failure that stimulated the release of EndoG together with cytochrome *c* and apoptosis inducing factor (AIF), and led to caspase-independent apoptosis in Leber's hereditary optic neuropathy cybrids.[9] Ischemia was shown to induce the release of EndoG from mitochondria in postnatal cardiomyocytes in the absence of caspase activation.[13] EndoG was seen translocated to cell nuclei in the mouse brain after transient focal ischemia.[7] Noise trauma induced nuclear translocation of EndoG and cell death in the inner ear.[16]

Our studies showed that EndoG is important in breast cancer and kidney cell injuries. In the confirmation of EndoG$-/-$ mouse viability, some human breast cancer cell lines live without expressing EndoG.[17] Cells which express EndoG are more sensitive to pro-apoptotic stimuli by etoposide or camptothecin treatments.[17] The hypoxia-reoxygenation of kidney tubular epithelial cells induces the leakage of EndoG from mitochondria which is regulated by endogenous ceramide production.[18] Again, this is similar to the role of DNase I; kidney tubular epithelial cells isolated from DNase I KO mice survived well, and were less sensitive to cisplatin treatment *in vitro* than WT cells.[19] In addition to this, our unpublished observations indicate that primary cells may lose their endonuclease activity in about 2 weeks after their removal from the body. Based on all of the above, we suggest that the reason the role of EndoG sometimes cannot be determined, particularly in embryonic tissues, is that it perhaps is not expressed there.

To test this, we compared the presence of EndoG protein in several tissues in mouse embryos and adult mice by using immunohistochemistry. Prior to the experiment, the specificity of antibody was tested on immortalized mouse kidney proximal tubules epithelial cells (TKPTS) pretreated with anti-EndoG siRNA and two human breast cancer cell lines, which are significantly different by EndoG expression.[17] As shown in Figure 1, high levels of EndoG were found in adult liver, heart, muscle and to a lesser extent, brain and kidney. These observations confirm previous findings regarding EndoG expression in these organs. In sharp contrast, EndoG had very little or no expression in adult spleen and in embryonic organs at the E13.5 stage. The least-differentiated mesenchymal cells, which are very often used for the isolation of embryonic fibroblasts and subsequent studies of cytotoxicity,[2,20] showed no detectable EndoG expression. Data presented in the NIH UniGene database (www.ncbi.nlm.nih.gov/UniGene) indirectly confirm that EndoG expression varies in different cell types, tissues and organs, and that embryos have very low (if any)

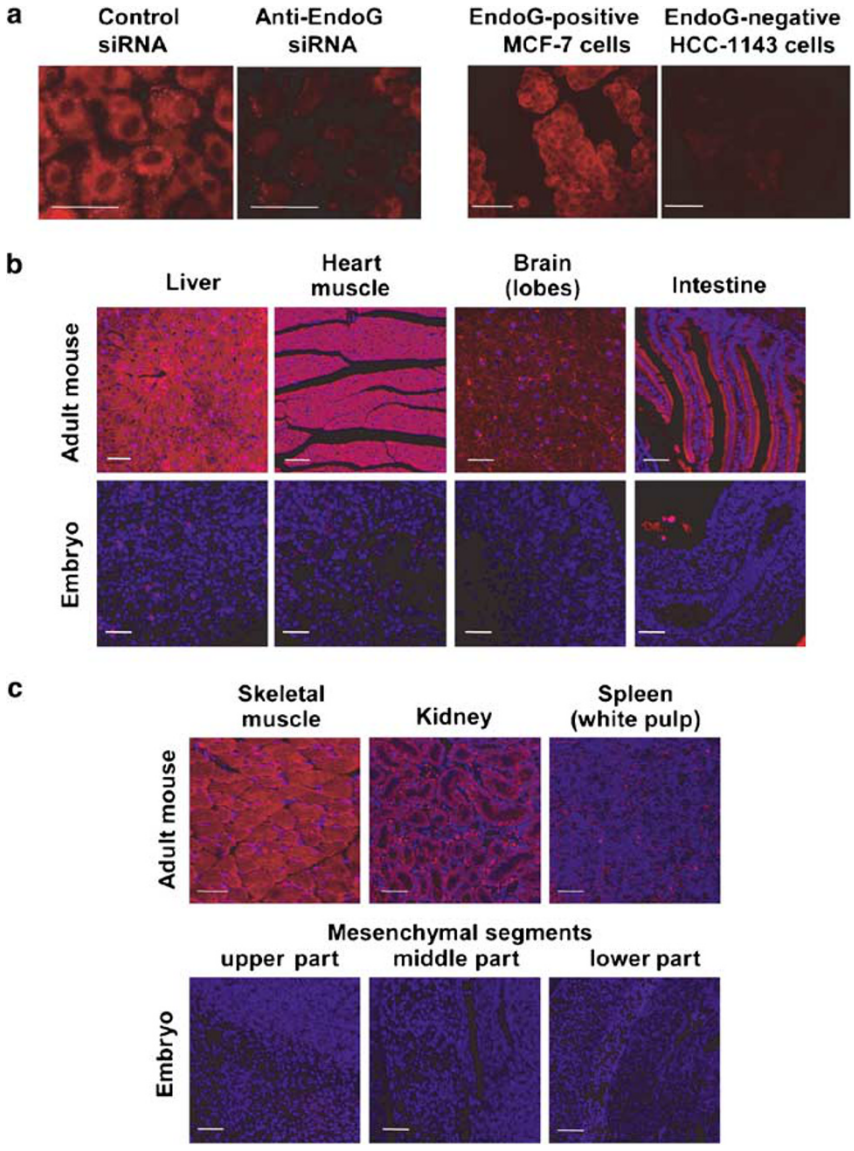

Figure 1 Reduced EndoG protein expression in embryonic mouse tissues compared to adult tissues. (**a**) The specificity of antibody has been evaluated using mouse renal tubular epithelial TKPTS cells pretreated with specific anti-EndoG siRNA (left panels) and two breast cancer cell lines with significant difference in EndoG expression (right panels). To knock down EndoG, experimental cells were transfected with siRNA (sense siRNA 5′-AUGCCUGGAACAACCUUGAdTdT-3′; antisense siRNA, 5′-UCAAGGUUGUUCCAGGCAUdTdT-3′), while control cells were transfected with control siRNA #1 (Dharmacon, Lafayette, CO, USA) at final concentration of 50 nM siRNA using the TransIT-TKO transfection reagent (Mirus, Houston, TX, USA) according to manufacturer's recommendations, in serum-free medium for at least 72 h. Two representative breast cancer cell lines were picked from the list of cell lines published previously by us.[17] In this study, the difference in EndoG expression was confirmed by real-time reverse transcriptase polymerase chain reaction, western blotting and enzymatic activity. Scale bar, 10 μm; (**b**) EndoG expression in organs of the adult mouse and corresponding mouse embryonic organs formed by the E13.5 stage. (**c**) EndoG expression of adult mouse tissues of mesenchymal origin and embryonic mesenchymal tissues. Although the embryo expresses a low level of EndoG; there is a noticeable heterogeneity between its expression in embryonic tissues in favor of the tissues which are already more differentiated (heart, liver, nervous system). The poorly differentiated tissues (mesenchymal segments) do not express EndoG protein. The analysis was performed using tissues from C57BL/6J mice (10 weeks old) and C57BL/6J embryos at the stage E13.5. After fixation with 10% neutral-buffered formalin, tissues were embedded and cut to 4 μm sections. For immunostaining, the sections were de-embedded, rehydrated and probed with polyclonal anti-EndoG antibody (Millipore, Billerica, MA, USA) at 1 : 200 dilution at $+4^{\circ}$C overnight. Secondary anti-rabbit-AlexaFluor 594 conjugate (Invitrogen, Carlsbad, CA, USA) at 1 : 400 dilution was applied for 1 h at room temperature. After subsequent washing, the sections were mounted under coverslips using the Prolong® Antifade kit with DAPI (Invitrogen). The sections were visualized using an Olympus IX-81 microscope (Olympus America Inc., Center Valley, PA, USA); images and acquisitions were made with a digital camera HAMAMATSU ORCA-ER (Hamamatsu Photonics K.K., Hamamatsu City, Japan) and software Slidebook 4.1 (SciTech Pty Ltd., Australia). Scale bar, 50 μm

expression of EndoG compared to adult organs. According to the database, EndoG is not expressed in mouse spleen as well.

In summary, the roles of EndoG in early development and postnatal cell injury seem to be very different. It is conceivable that while EndoG seems, at least in some models, to be non-

essential for embryonic development, it is very important for injury to adult cells.

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