Data input form: MouseAGE Database

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Preferred acknowledgement:

Summarise supporting data attached:

The figure, embedded in the form, summarises the cross-breeding scheme between p66shc KO mice (provided by Prof. Piergiuseppe Pelicci, IEO, Milan, Italy) and SOD2 KO mice (originally provided by Prof. CJ Epstein, UCSF); the accompanying table displays genotype distribution in the progeny at weaning (3 weeks), which reveals no rescue of SOD2 KO early lethality by p66shc deletion.

Ageing / disease:

Mitochondrial dysfunction, dilated cardiomyopathy, early lethality

Model:

MnSOD (SOD2) deficient mice (C57BL6/J); p66shc KO mice (129/Sv)

Clinically relevant? (Yes or No):

Yes

Explanation of why clinically relevant or not:

Mitochondrial oxidative stress and damage, as modelled by genetic MnSOD (Manganese dependent Superoxide Dismutase, or SOD2) inactivation(1), are well recognized driving causes for human myocardiopathy related to age or exposure to anticancer drugs like doxorubicin(2). Accordingly, the mitochondrial superoxide dismutase A16V polymorphism predisposes human beings to the cardiomyopathy associated with hereditary haemochromatosis(3). In more general terms, SOD2 KO mice model the accumulation of mitochondrial oxidative damage as occurs as one of the hallmarks of ageing(4).

Conversely, p66KO mice display marked tissue resistance to oxidative stress and to several ROS and age-related human diseases including atherosclerosis, limb ischemia, diabetes/insulin resistance and neurodegeneration(5–7). Initial claims of enhanced lifespan in this mouse strain have however been questioned by more detailed studies(8).

Characteristics, timing of appearance of phenotype and phenotypic tests used (attach supporting data when unpublished if available):

SOD2 KO mice display a severe mitochondrial phenotype with prenatal/neonatal lethality, dilated cardiomyopathy, accumulation of lipid in liver and skeletal muscle and metabolic acidosis, especially in the B6 genetic background (mortality between E15 and P1)(1,9).

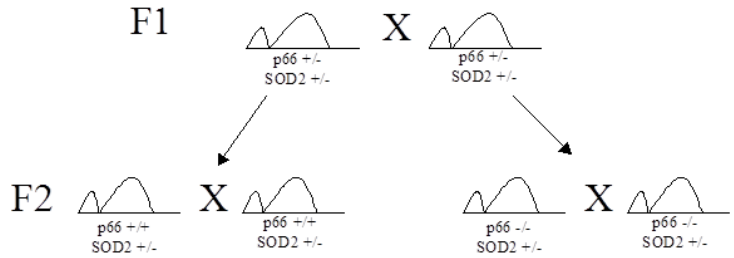
P66shc KO mice are phenotypically normal and fertile, but reportedly leaner than WT controls(7).

If applicable, Intervention(s) performed (type, dose, route of administration, frequency):

We reasoned that genetic deletion of p66 might, by reducing oxidative mitochondrial burden and tissue damage, rescue the SOD2-null phenotype. We therefore cross-bred the two strains so as to generate p66 +/+ and p66 -/- mice of the three (SOD2 +/+, SOD2 +/- and SOD2 -/-) POSSIBLE SOD2 genotypes. Note that the two strains were on different genetic backgrounds (B6 for SOD2 and 129/Sv for p66 KO).

Phenotype post-treatment (include null or negative results and attach supporting data separately):

SOD2 genotype distribution was similar in the p66WT and p66 KO lineages. At weaning, no SOD2 -/- pups were detected, irrespective of p66 genotype, indicating no major rescue of SOD2 KO lethality by p66shc deletion (see attached table). Death rates during the first week post-partum were comparable between the two strains, while E13 SOD2 -/- embryos were found at the expected mendelian rate in the uterus of both p66 +/+ and p66KO mothers (not shown). No further biochemical or histopathological analyses were performed on pups found dead or on SOD +/- animals.



F3

	Shc p66+/+	Shc p66 -/-	Tot
SOD2 +/+	11	12	23
SOD2 +/-	23	22	45
SOD2 -/-	0	0	0
Tot	34	34	68

Comment.

The above unpublished pilot study does not support the hypothesis of p66shc contributing to mitochondrial oxidative stress in the context of SOD2 deficiency. This result was rather unexpected based on the vast literature on p66shc prooxidant/proapoptotic roles and involvement in ROS-dependent diseases both in human and animal models. However, early lethality may not represent a sufficiently sensitive end-point for assessing SOD2-p66shc functional interaction. Moreover, genetic background effects were not sufficiently controlled in this experiment(9).

It is also possible that SOD2 is epistatic to p66shc prooxidant action, i.e. that beneficial effects of p66sh deletion require MnSOD(10). In keeping with this idea is evidence that p66shc inhibits Foxo3a, a major SOD2 transcriptional regulator(11,12), and our preliminary studies revealing upregulation of MnSOD in p66 KO Mouse embryonic fibroblasts (MEFs), but not in adult heart or liver, compared to WT controls (not shown). This alternative hypothesis deserves further investigation.

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