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A High Glycolytic Flux Supports the Proliferative Potential of Murine Embryonic Stem Cells

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ABSTRACT

Embryonic stem (ES) cells are immortal and present the ability to self-renew while retaining their ability to differentiate. In contrast, most primary cells possess a limited proliferative potential, and when this is exhausted, undergo an irreversible growth arrest termed senescence. In primary cells, senescence can be also triggered by a variety of stress to which ES cells are highly refractory. Here the authors report that the proliferative capacity of murine ES cells closely correlates with high activity of different glycolytic enzymes, elevated glycolytic flux, and low mitochondrial oxygen consumption. The direct relation between glycolytic flux and the ability of ES cells to proliferate is further remarked in experiments where glycolysis or ES cell self-renewal was specifically inhibited. It was previously reported that the upregulation of glycolysis in primary cells results in life span extension. The authors hypothesize that the naturally high glycolytic flux observed in murine ES cells can be responsible for their unlimited proliferative potential. *Antioxid. Redox Signal.* 9, 293–299.

INTRODUCTION

Most somatic cells present a limited proliferative potential under standard tissue culture conditions. They undergo a permanent cell cycle arrest, termed senescence upon reaching replicative exhaustion (replicative senescence) or induced by several stress (stress-induced senescence) (16). Although both replicative and stress-induced senescence (SIS) are phenotypically quite similar to each other, their underlying mechanisms are quite different (37). Replicative senescence is mainly caused by telomere erosion at chromosome end as a result of cell division, which can be bypassed by the ectopic expression of telomerase in human primary fibroblasts (4, 39). In contrast, SIS can be triggered in cells of virtually any age by the expression of active oncogenes, cycline-dependent kinase (CDK) inhibitors, mild oxidative stress, and other stimuli (26, 27, 29). Thus, SIS is recognized as telomere-independent senescence.

In striking contrast with the other primary cells, both murine and human embryonic stem (ES) cells are immortal under standard tissue culture conditions (32, 33). ES cells show intrinsic telomerase activity, which is lacking in most of primary cells. But the immortality of ES cells can not be solely explained by its telomerase activity, as ES cells are also resistant to SIS, telomere-independent senescence.

Murine cells are one of the best systems to investigate the mechanisms behind SIS. Normal laboratory mice display ex-

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ceptionally long telomeres, yet mouse embryo fibroblasts (MEFs) with intact telomerase activity generally have a life span of only 10–20 population doublings under normal culture conditions (30). This senescence response observed in MEFs has been attributed to 'culture shock' (31) and is not observed in murine ES cells, which argues for additional mechanisms besides telomere regulation involved in ES cells immortality. Possible factors contributing to this 'culture shock' are, amongst others, growth on plastic, and constant exposure to mitogenic factors from serum, or high oxygen levels. The modification of tissue culture conditions to avoid these stress such as the use of serum-free medium (24) or culture in low oxygen conditions (28) results in an extension of the cellular life span of murine or human primary fibroblasts (18). Despite our current knowledge of the stimuli and molecular mechanisms governing senescence, there is still a poor understanding of why ES cells are significantly refractory to SIS.

Recently we reported that increased glycolysis results in an extension of cellular life span in MEFs (23). Conversely, inhibition of glycolysis induces premature senescence. We discovered that enhanced glycolysis modulates the cellular life span concomitant with the reduction of oxidative damage. Thus, enhanced glycolysis can render murine fibroblasts resistant to SIS. Interestingly, glycolytic flux is impaired through the senescence process under standard culture condition. As phosphoglycerate mutase (PGM), one of the glycolytic enzymes, is post-transcriptionally regulated by p53 status (23), glycolysis might be regulated in a cellular-context dependent manner, senescence or not. Moreover, elevated glycolysis at high oxygen concentration is one of the most characteristic properties of cancer cells, known as the Warburg effect (36). As senescence is a safeguard mechanism against tumorigenesis (5, 10, 11, 29), elevated glycolysis could play an essential role not only in the cellular adaptation to hypoxic conditions in tumors, but also in contributing to bypass senescence at an early stage of tumorigenesis (22). Previously, we have also reported that murine ES cells naturally display high glycolysis (23). Here we investigate the impact of the glycolytic flux on the proliferative capacity of murine ES cells. Our results suggest that the naturally high glycolytic flux observed in ES cells contributes to maintain the proliferative capacity of murine ES cells.

MATERIALS AND METHODS

Cell culture and retroviral infection

Mouse embryo fibroblasts (MEFs) were isolated from 13.5 day postcoitum embryos of C57BL/6 mice, as described previously (8). MEFs were grown in DMEM supplemented with 10% FCS. Mouse ES cell lines CGR8 (feeder independent) and DE3 and RW4 (feeder dependent cells) were grown as described by Smith *et al.* (32). MEF feeder cells were prepared as previously described (32). To induce differentiation, ES cells were switched to medium without leukemia inhibitory factor (LIF). 2-Deoxyglucose (2-DG) treatment was maintained for 3 days at the indicated concentrations. To investigate the effect of low oxygen, cells were cultured in a hypoxic chamber (Coy Laboratories, Grasslake, MI) at 3% oxygen.

All retroviruses were produced by transfecting the relevant plasmid DNA into the LinXE packaging cell line (from the laboratory of Dr. David Beach) (14), and infections were conducted in the presence of 8 μg/ml polybrene. Infected cells were selected in hygromycin (75 μg/ml). After 8–10 days of selection, cultures were propagated according to a 3T3 protocol as described previously (8). Briefly, every 3 days, cells were trypsinized and replated at a density of 10^6 cells per 10 cm plate.

Glycolytic flux measurement and enzymatic assays

The activities of several glycolytic enzymes [glyceraldehyde-3-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase (PGK), phosphoglycerate mutase (PGM), and enolase (ENO)] were measured spectrophotometrically as described (3). To determine glycolytic flux, we measured the metabolism of d-[3–3H]glucose into water after the triose phosphate isomerase reaction as described (35). All the values were normalized by protein content.

Measurement of oxidative damage

Oxidative DNA damage was assessed using an immunohistochemical assay as described (13, 20) with minor modifications. Samples (0.5 μg) of genomic DNA were dot-blotted onto Hybond-XL membrane (Amersham, UK). After baking at 80°C for 5 min, the membrane was probed with a mouse anti-8-hydroxyguanine antibody (Trevigen, UK) (34) and DNA loading was monitored by staining with ethidium bromide. The signal was quantified by NIH image software and normalized against ethidium bromide staining.

Measurement of mitochondrial respiration

Respiration measurements were conducted using a Clark-type O_2 electrode (Rank Bros., Cambridge, UK) with samples containing 3.6×10^6 cells. The rate of O_2 consumption was calculated from the difference in respiration before and after the addition of 500 n*M* myxothiazol, an inhibitor of mitochondrial complex III (1).

RESULTS

Elevated glycolysis in murine ES cells

As shown in Fig. 1A, MEFs stop proliferating after 8–10 passages in cell culture. At this point they reach senescence, as observed by their flat enlarged cell appearance characteristic of senescent cells (Fig. 1B). This growth arrest involves upregulation of p53 activity and can be avoided by the expression of a p53 dominant-negative allele (p53DN, Fig. 1A). MEFs immortalized by p53DN expression are spindle shaped and small (Fig. 1B). The growth kinetics of control MEFs are in sharp contrast with those of murine ES cells that proliferate for months under standard tissue culture conditions (20% oxygen) without any sign of replicative exhaustion (Fig. 1A). In addition, CGR8 murine ES cells duplicate much faster than p53DN expressing MEFs or wild-type MEFs at early passages (Fig. 1A and data not shown). Previously we re-

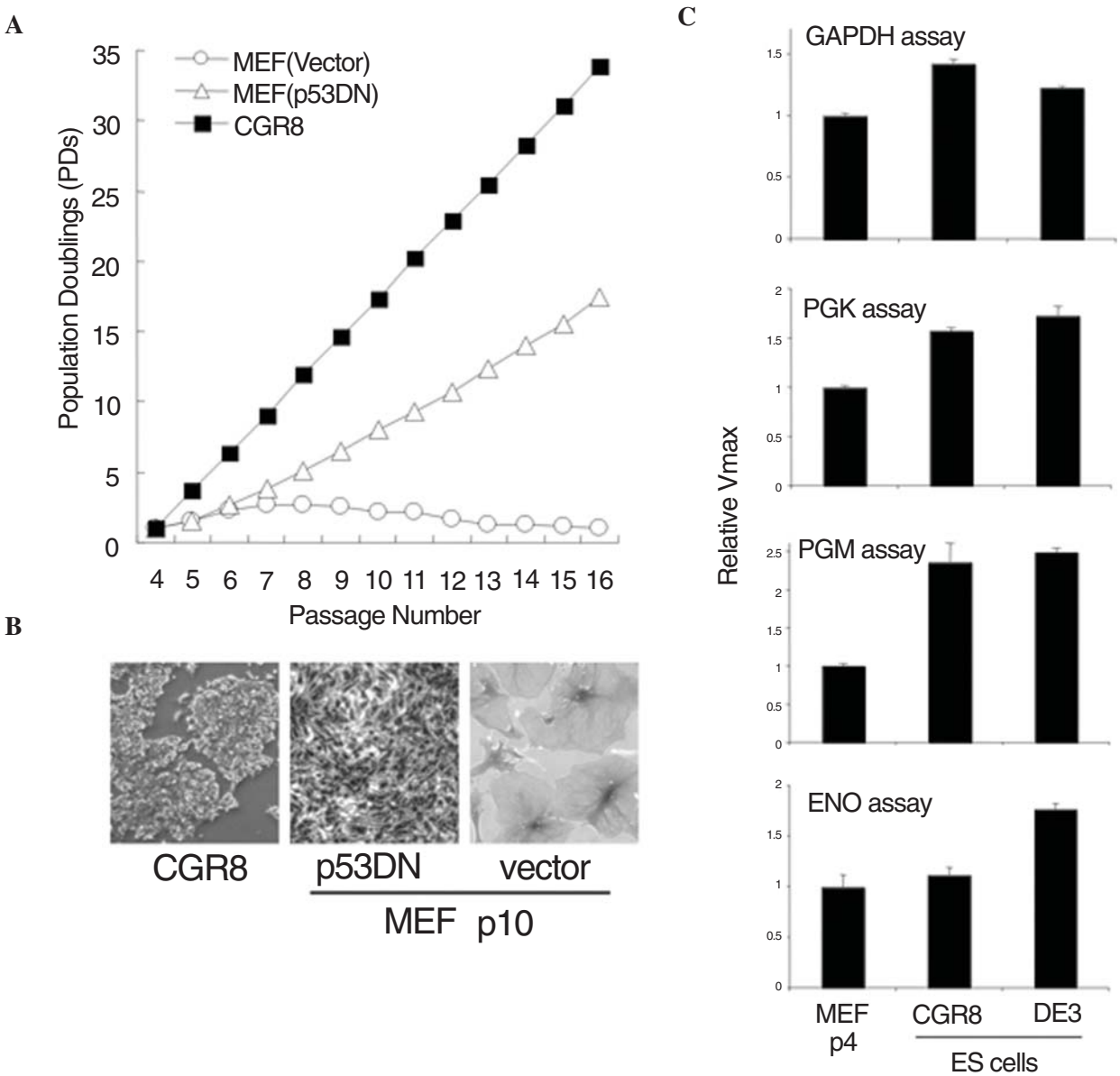

FIG. 1. Murine ES cells are immortal and display high activities of several glycolytic enzymes. (**A**) Growth curves comparing the replicative potential of murine ES cells and MEFs. Standard 3T3 protocols were conducted for murine ES cells CGR8 (*closed squares*), p53DN-expressing MEFs (*open triangles*), and vector-bearing MEFs as control (*open circles*). (**B**) Representative microphotographs of CGR8 murine ES cell line (*left panel*), p53DN-expressing MEFs (*middle panel*), and vector-bearing MEFs (*right panel*) at passage 10 are shown. (**C**) The activity of several glycolytic enzymes was measured in the indicated cells. Enzymatic activity is shown as relative Vmax, normalized to the value of MEFs at passage 4. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PGK, phosphoglycerate kinase; PGM, phosphoglycerate mutase; ENO, enolase.

ported that murine ES cells display a much higher glycolytic flux than MEFs immortalized by p53 inactivation or wild-type MEFs at very early passage (23). To further understand why murine ES cells exhibit enhanced glycolysis, we compared the activities of several glycolytic enzymes in wild-type MEFs at passage 4 and the CGR8 and DE3 murine ES cells. We measured the activities of GAPDH, PGK, PGM, and ENO, as described in Materials and Methods. We confirmed that ES cells present a high level of PGM activity as reported previously (23). In addition, as shown in Fig. 1C, the activities of GAPDH, PGK, and ENO were between 1.2–1.7-fold higher in ES cells than in MEFs. We also observed that MEFs immortalized by p53DN expression presented enzymatic activities comparable to those of MEFs at early passages (data not shown). From the above described experiments, we conclude that the elevated proliferation observed in ES cells correlates with high activities of different glycolytic enzymes.

Low level of mitochondrial respiration in murine ES cells

As we reported previously, enhanced glycolysis can extend the life span of MEFs and correlates with a reduced production of reactive oxygen species (ROS). It is well known that the majority of ROS produced in the cells are generated in the mitochondria (2). Therefore, we decided to measure the rate of mitochondrial respiration and its relation with glycolysis and cell proliferation. A comparison of the oxygen consumption between proliferating (early passage) and senescent MEFs showed that oxygen consumption increases by twofold at late passage (Figs. 2A and B). Conversely, MEFs immortalized via the ectopic expression of p53DN or PGM showed less oxygen consumption than control senescent MEFs at equivalent passage (passage 10). These MEFs display a higher glycolytic flux than senescent cells, as we reported previously (23). Since murine ES cells also present a high glycolytic flux and elevated activity of different glycolytic enzymes, next we decided to evaluate their rate of mitochondrial respiration. We showed that murine ES cells present extremely low oxygen consumption, much lower than that of wild-type MEFs at early passages. In summary, we found that murine ES cells exhibit a characteristic metabolic profile shared with proliferative primary cells, consistent with an elevated glycolysis and reduced mitochondrial oxygen consumption.

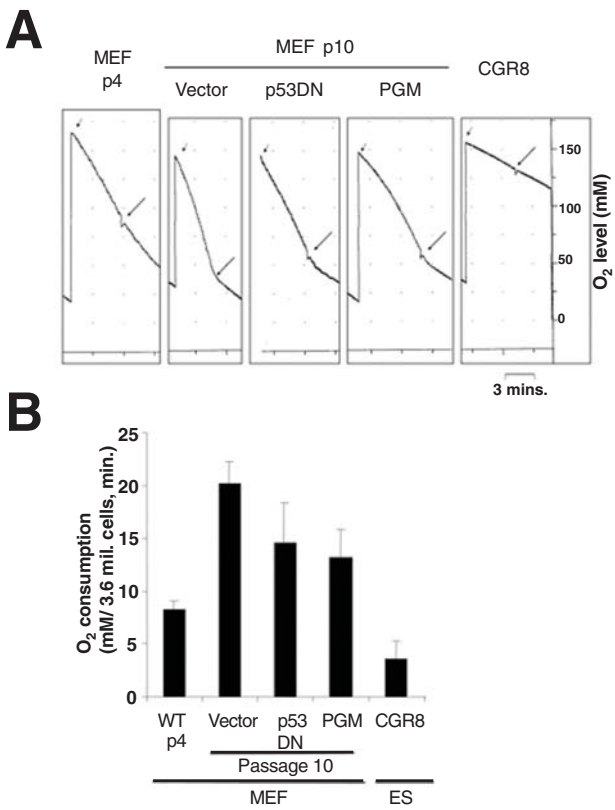

FIG. 2. Mitochondrial oxygen consumption is low in murine ES cells. (**A**) Representative oxygen consumption curves for the indicated cells. *Arrowheads* signal to the starting point of analysis, while *arrows* indicate the point where myxothiazol was added. (**B**) Oxygen consumption in ES cells and various MEFs. Average values of three independent experiments for oxygen consumption are shown.

Murine ES cells are resistant to oxidative damage

It has been reported that normal levels of oxygen (20% oxygen) are detrimental for the life span of primary fibroblasts, as a consequence of the accumulation of oxidative damage. Conversely, culture in low oxygen prevents the accumulation of oxidative damage and contributes to restoration of the full proliferative potential of human and murine primary fibroblasts (18, 28).

As the elevated glycolytic rate of ES cells closely correlates with their proliferative capacity, we measured the glycolytic flux and oxidative damage of murine ES cells cultured in 3% or 20% oxygen. It is well known that culture in low oxygen conditions can increase glycolytic flux. This is known as the Pasteur effect (17). In passage 3 MEFs, the glycolytic flux observed under 3% oxygen is about twofold higher than that observed under 20% oxygen. In CGR8 cells, culture in low oxygen conditions also increases the glycolytic flux by about 30% compared with 20% oxygen culture. Noteworthy, the glycolytic flux of murine ES cells grown in 20% oxygen is higher than that of MEFs grown under low oxygen (Fig. 3A). These results suggest that ES cells can maintain elevated glycolytic flux even under 20% oxygen.

To evaluate the correlation between the mitochondrial respiration, oxidative damage and proliferative potential, we decided to measure the oxidative damage accumulated in MEFs and ES cells grown under 3% or 20% oxygen. 8-Hydroxy-2′-deoxyguanosine (8-OHdG) is one of the most common adducts present in DNA as a consequence of oxidative damage. The senescent cells accumulate high levels of 8-OHdG, while the immortalized cells suffer fewer oxidative damage in their genomes (23, 28). We confirmed those results by blotting the genomic DNA from MEFs cultured under different oxygen concentrations and probing them with an anti 8-OHdG antibody (Fig. 3B). In addition, PGM expressing MEFs grown in 20% oxygen culture exhibit less oxidative damage than vector-bearing MEFs grown in 20% oxygen, consistent with our previous findings (23). Finally, we also found that the levels of 8-OHdG in murine ES cells are almost similar to that of immortalized MEFs grown in 20% oxygen and wild-type MEFs in 3% oxygen. We concluded that the genome of murine ES cells is resistant from oxidative damage even in 20% oxygen conditions to which MEFs genome is quite sensitive.

The glycolytic rate of murine ES cells correlates with their proliferative potential

To evaluate the impact of the high glycolytic flux of murine ES cells on their proliferative potential, we took two different approaches. First, we analyzed the effect of inhibiting glycolysis in murine ES cells. Second, we measured glycolytic flux during the process of spontaneous differentiation of murine ES cells, where proliferation rate declines sharply.

2-Deoxyglucose (2-DG) is a nonmetabolizable glucose analogue that blocks glycolysis at the phosphohexose iso-

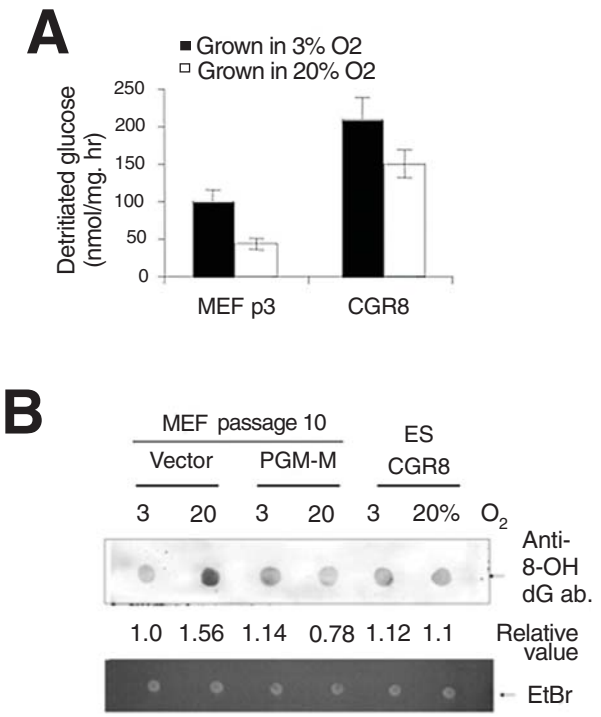

FIG. 3. Murine ES cells display high glycolysis under atmospheric oxygen conditions. (**A**) Glycolytic flux of murine ES cells and MEFs at passage 3. Cells were cultured under two different oxygen conditions, 20% oxygen (*open bars*) and 3% oxygen (*closed bars*). (**B**) Dot blot analysis of 8-OHdG to measure the oxidative damage in the genome. The indicated cells were cultured under 3% or 20% oxygen condition. Genomic DNA of these cells was prepared, dot-blotted, and probed with an anti-8-OHdG antibody. Signal intensity was normalized to the ethidium bromide signal. Relative values of signal intensity are shown, compared to vector-bearing MEFs grown in 3% oxygen culture as control. PGM expressing MEFs and CGR8 are known to show higher glycolytic flux and PGM activity than control (Ref. 23 and Fig.1).

merase step and has been frequently used as a glucose starvation mimetic. Initially, we confirmed that a low concentration of 2-DG (15 m*M*) had a negligible effect on the viability of MEF feeder cells for 3 days. However, in murine feeder dependent ES cells (RW4), treatment with 5 and 15 m*M* 2-DG results in a half of ES cell number and a complete halt of ES cells proliferation after 24 h, respectively (Fig. 4A). Three days after treatment, massive cell death of ES cells is observed (data not shown). These effects are feeder-cell independent, as the CGR8 ES cells (feeder-independent ES cells) showed similar sensitivity to glycolytic inhibition (Fig. 4B). Therefore, murine ES cells displayed an increased sensitivity to glycolytic inhibition when compared with MEFs.

Next, we measured the glycolytic flux during the differentiation of murine ES cells induced by the withdrawal of LIF from their culture medium. The cytokine LIF is essential for the maintenance of the stem cell characteristics of murine ES cells. Upon LIF withdrawal from culture medium, cells stop growing (Fig. 4C, left panel). The differentiated ES cells exhibit a fibroblast-like morphology when compared with the usual small colonies formed by murine ES cells (data not shown). Interestingly, differentiating ES cells showed a lower glycolytic flux, corresponding to less than half of that observed in proliferating ES cells (Fig. 4C, right panel). These data suggest that the elevated enhanced glycolytic of ES cells is directly related to their proliferative potential.

DISCUSSION

In the present report, we found a close correlation between the glycolytic flux and proliferative potential of murine ES cells. Several lines of evidence highlight this. First, ES cells posses a higher replicative potential and a higher glycolytic flux than that of immortalized MEFs or wild-type MEFs at early passages. Second, ES cells are more sensitive to glycolytic inhibition than MEFs are. Third, upon ES cells differentiation, the glycolytic flux of ES cells drops dramatically.

We recently reported that enhanced glycolysis could extend the life span of MEFs correlating with reduced levels of oxidative damage. Ectopic expression of the glycolytic enzyme PGM renders MEFs resistant to oxidative stress and enables them to bypass senescence. Noteworthy among several glycolytic enzymes, PGM activity is most prominently enhanced in ES cells when compared with MEFs. Murine ES cells are also known to be more resistant to oxidative stress than MEFs (9, 23). Here we unveiled that murine ES cells maintain high glycolytic flux even under standard culture conditions and are resistant to oxidative damage caused by 20% oxygen. As PGM expressing MEFs also displayed reduced oxidative damage and enhanced glycolysis, it can be suggested that enhanced glycolysis might protect murine ES cells from senescence triggered by oxidative damage.

How can enhanced glycolysis protect cells from oxidative damage? Murine ES cells use less mitochondrial oxygen than MEFs. In fact, ES cells present a characteristic metabolic fingerprint similar to that of primary cells; enhanced glycolysis with reduced mitochondrial oxygen consumption. Little is known about how these metabolic properties can be concerted in ES cells. Recently, it has been suggested that mitochondrial function is regulated partly by the transcriptional factor hypoxia inducing factor 1 (HIF-1) (21). As HIF-1 can also upregulate most of the glycolytic enzymes at the transcriptional level, HIF-1 might control glycolysis and mitochondrial respiration in a concerted manner. However, the enhanced glycolysis and reduced oxygen consumption of murine ES cells cannot be only explained according to HIF-1 levels. For example, PGM is the only glycolytic enzyme that is not regulated by HIF-1 (19), and ES cells present high levels of PGM activity.

Interestingly mitochondrial respiration can be repressed by p53 inactivation *in vitro* (in this report) and *in vivo* (25). As p53 can also affect glycolytic capacity (12, 23), apoptotic response (38), and immortalization of primary cells (7); the modulation of the p53 pathway in ES cells could be behind their unique metabolic properties (6). It is also well known that p53 inactivation is one of the most common genetic alterations in cancer cells. There are not apparent genetic defects in ES cells, as they are the diploid primary cells that maintain

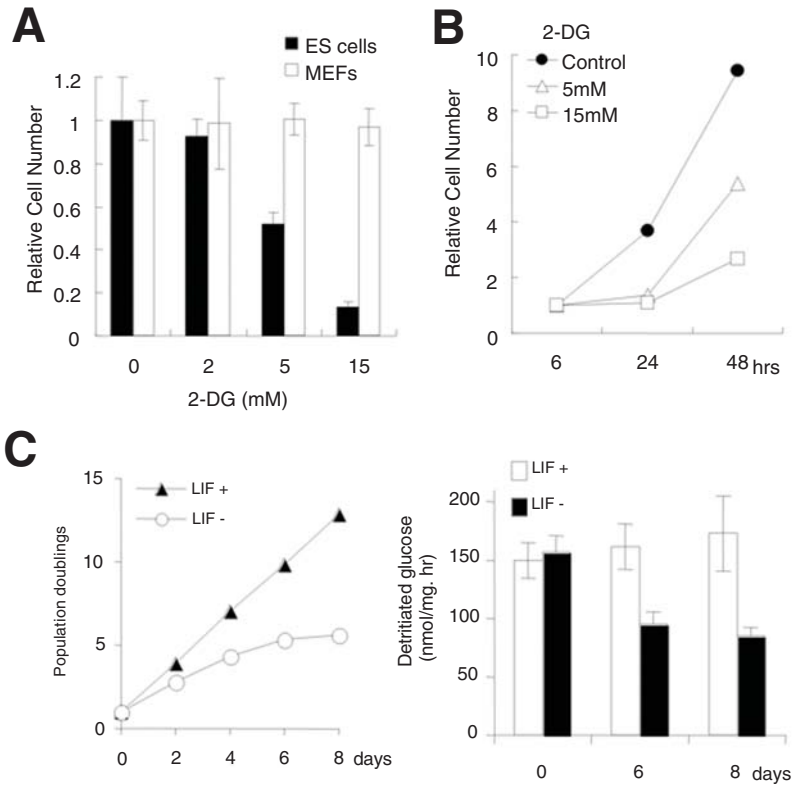

FIG. 4. The glycolytic flux of murine ES cells correlates with its proliferative capacity. (**A**) Glycolytic inhibition reduces the proliferative potential of murine ES cells. Feeder dependent ES cells (RW4) or MEFs were treated with 2-DG at the indicated concentrations for 3 days. The graph shows the relative cell numbers after normalization to the values of nontreated cells. (**B**) Representative growth curves of feeder independent ES cells (CGR8) after 2-DG treatment. The graph shows the relative cell numbers after normalization to the values at the moment of 2-DG addition (6 h after plating the cells). (**C**) Effects of LIF withdrawal over the growth of CGR8 cells (*left panel)*. Effects of LIF withdrawal over the glycolytic flux of CGR8 cells (*right panel)*. Glycolytic flux measured in cells growing in the presence of LIF (*open bars*) or in cells cultured in the medium without LIF (*closed bars*).

both their self-renewal and differentiating capacity. Consequently, the p53 function might be blunted in ES cells in a different manner from cancer cells, possibly through transient epigenetic regulations, which would be consistent with the heavy epigenetic control operating in ES cells (15).

In this sense, further research on the mechanisms underlying the immortality of ES cells might unveil novel molecular pathways involved also in cancer progression. Indeed, increasing attention has recently been drawn to cancer stem cells, which show stem cell characteristics that can drive cancer proliferation and progression. The findings presented here suggest that the Warburg effect, initially discovered in cancer cells, might also affect the proliferative capacity of ES cells.

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ABBREVIATIONS

2-DG, 2-deoxyglucose; ENO, enolase; ES cells, embryonic stem cells; FCS, fetal calf serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HIF-1, hypoxia inducing factor 1; LIF, leukemia inhibitory factor; MEFs, mouse embryonic fibroblasts; p53DN, p53 dominant negative allele; PGK, phosphoglycerate kinase; PGM, phosphoglycerate mutase; ROS, reactive oxygen species; SIS, stress-induced senescence.

REFERENCES

1. Almeida A, Almeida J, Bolanos JP, and Moncada S. Different responses of astrocytes and neurons to nitric oxide: the role of glycolytically generated ATP in astrocyte protection. *Proc Natl Acad Sci USA* 98: 15294–15299, 2001.
2. Beckman KB and Ames BN. The free radical theory of aging matures. *Physiol Rev* 78: 547–581, 1998.
3. Beutler E (Ed.). Glycolytic enzymes. In: *Methods in Haematology.* Edinburgh and New York: Churchill Livingston, 1986, pp. 57–72.
4. Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichtsteiner S, and Wright WE. Extension of life-span by introduction of telomerase into normal human cells. *Science* 279: 349–352, 1998.
5. Braig M, Lee S, Loddenkemper C, Rudolph C, Peters AH, Schlegelberger B, Stein H, Dorken B, Jenuwein T, and Schmitt CA. Oncogene-induced senescence as an initial barrier in lymphoma development. *Nature* 436: 660–605, 2005.
6. Burdon T, Smith A, and Savatier P. Signalling, cell cycle and pluripotency in embryonic stem cells. *Trends Cell Biol* 12: 432–438, 2002.
7. Campisi J. Cellular senescence as a tumor-suppressor mechanism. *Trends Cell Biol* 11: S27–S31, 2001.
8. Carnero A, Hudson JD, Price CM, and Beach DH. p16INK4A and p19ARF act in overlapping pathways in cellular immortalization. *Nat Cell Biol* 2: 148–155, 2000.
9. Castro–Obregon S and Covarrubias L. Role of retinoic acid and oxidative stress in embryonic stem cell death and neuronal differentiation. *FEBS Lett* 381: 93–97, 1996.
10. Chen Z, Trotman LC, Shaffer D, Lin HK, Dotan ZA, Niki M, Koutcher JA, Scher HI, Ludwig T, Gerald W, Cordon–Cardo C, and Pandolfi PP. Crucial role of p53-dependent cellular senes-

cence in suppression of Pten-deficient tumorigenesis. *Nature* 436: 725–730, 2005.
11. Collado M, Gil J, Efeyan A, Guerra C, Schuhmacher AJ, Barradas M, Benguria A, Zaballos A, Flores JM, Barbacid M, Beach D, and Serrano M. Tumour biology: senescence in premalignant tumours. *Nature* 436: 642, 2005.
12. Dang CV and Semenza GL. Oncogenic alterations of metabolism. *Trends in Biochem Sci* 24: 68–72, 1999.
13. Esashi F and Yanagida M. Cdc2 phosphorylation of Crb2 is required for reestablishing cell cycle progression after the damage checkpoint. *Mol Cell* 4: 167–74, 1999.
14. Hannon GJ, Sun P, Carnero A, Xie LY, Maestro R, Conklin DS, and Beach D. MaRX: an approach to genetics in mammalian cells. *Science* 283: 1129–1130, 1999.
15. Hattori N, Nishino K, Ko YG, Hattori N, Ohgane J, Tanaka S, and Shiota K. Epigenetic control of mouse Oct-4 gene expression in embryonic stem cells and trophoblast stem cells. *J Biol Chem* 279: 17063–17069, 2004.
16. Hayflick L and Moorhead PS. The serial cultivation of human diploid cell strains. *Exp Cell Res* 25: 585–621, 1961.
17. Hochachka PW. Patterns of O2-dependence of metabolism. *Adv Exp Med Biol* 222: 143–151, 1988.
18. Itahana K, Zou Y, Itahana Y, Martinez JL, Beausejour C, Jacobs JJ, Van Lohuizen M, Band V, Campisi J, and Dimri GP. Control of the replicative lifespan of human fibroblasts by p16 and the polycomb protein Bmi-1. *Mol Cell Biol* 23: 389–401, 2003.
19. Iyer NV, Kotch LE, Agani F, Leung SW, Laughner E, Wenger RH, Gassmann M, Gearhart JD, Lawler AM, Yu AY, and Semenza GL. Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha. *Genes Dev* 12: 149–162, 1998.
20. Katayama S, Kitamura K, Lehmann A, Nikaido O, and Toda T. Fission yeast F-box protein Pof3 is required for genome integrity and telomere function. *Mol Biol Cell* 13: 211–224, 2002.
21. Kim JW, Tchernyshyov I, Semenza GL, and Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab* 3: 177–185, 2006.
22. Kondoh H, Lleonart ME, Gil J, Beach D, and Peters G. Glycolysis and cellular immortalization. *Drug Disc Today* 2: 263–267, 2005.
23. Kondoh H, Lleonart ME, Gil J, Wang J, Degan P, Peters G, Martinez D, Carnero A, and Beach D. Glycolytic enzymes can modulate cellular lifespan. *Cancer Res* 65: 177–185, 2005.
24. Loo DT, Fuquay JI, Rawson CL, and Barnes DW. Extended culture of mouse embryo cells without senescence: inhibition by serum. *Science* 236: 200–202, 1987.
25. Matoba S, Kang JG, Patino WD, Wragg A, Boehm M, Gavrilova O, Hurley PJ, Bunz F, and Hwang PM. p53 regulates mitochondrial respiration. *Science* 312: 1650–1653, 2006.
26. McConnell BB, Starborg M, Brookes S, and Peters G. Inhibitors of cyclin-dependent kinases induce features of replicative senescence in early passage human diploid fibroblasts. *Curr Biol* 8: 351–354, 1998.
27. Ogryzko VV, Hirai TH, Russanova VR, Barbie DA, and Howard BH. Human fibroblast commitment to a senescence-like state in response to histone deacetylase inhibitors is cell cycle dependent. *Mol Cell Biol* 16: 5210–5218, 1996.
28. Parrinello S, Samper E, Krtolica A, Goldstein J, Melov S, and Campisi J. Oxygen sensitivity severely limits the replicative lifespan of murine fibroblasts. *Nat Cell Biol* 5: 741–747, 2003.
29. Serrano M, Lin AW, McCurrach ME, Beach D, and Lowe SW. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell* 88: 593–602, 1997.
30. Serrano M and Blasco MA. Putting the stress on senescence. *Curr Opin Cell Biol* 13: 748–753, 2001.
31. Sherr CJ and DePinho RA. Cellular senescence: mitotic clock or culture shock? *Cell* 102: 407–410, 2000.
32. Smith AG, Heath JK, Donaldson DD, Wong GG, Moreau J, Stahl M, and Rogers D. Inhibition of pluripotential embryonic stem cell differentiation by purified polypeptides. *Nature* 336: 688–690, 1988.
33. Thomson JA, Itskovitz–Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, and Jones JM. Embryonic stem cell lines derived from human blastocysts. *Science* 282: 1145–1147, 1998.
34. Trinei M, Giorgio M, Cicalese A, Barozzi S, Ventura A, Migliaccio E, Milia E, Padura IM, Raker VA, Maccarana M, Petronilli V, Minucci S, Bernardi P, Lanfrancone L, and Pelicci PG. A p53-p66Shc signalling pathway controls intracellular redox status, levels of oxidation-damaged DNA and oxidative stress-induced apoptosis. *Oncogene* 21: 3872–3878, 2002.
35. Urbano AM, Gillham H, Groner Y, and Brindle KM. Effects of overexpression of the liver subunit of 6-phosphofructo-1-kinase on the metabolism of a cultured mammalian cell line. *Biochem J* 352: 921–927, 2000.
36. Warburg O. *The Metabolism of Tumours*. Constable London, 1930.
37. Wright WE and Shay JW. Historical claims and current interpretations of replicative aging. *Nat Biotechnol* 20: 682–688, 2002.
38. Xu D, Wilson TJ, Chan D, De Luca E, Zhou J, Hertzog PJ, and Kola I. Ets1 is required for p53 transcriptional activity in UV-induced apoptosis in embryonic stem cells. *EMBO J* 21: 4081–4093, 2002.
39. Zakian VA. Telomeres: beginning to understand the end. *Science* 270: 1601–1607, 1995.

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1. Susana Solá, Ana L. Morgado, Cecília M.P. Rodrigues. 2012. Death Receptors and Mitochondria: Two Prime Triggers of Neural Apoptosis and Differentiation. *Biochimica et Biophysica Acta (BBA) - General Subjects* . [CrossRef]
2. Elisia D. Tichy, Resmi Pillai, Li Deng, Jay A. Tischfield, Philip Hexley, George F. Babcock, Peter J. Stambrook. 2012. The abundance of Rad51 protein in mouse embryonic stem cells is regulated at multiple levels. *Stem Cell Research* **9**:2, 124-134. [CrossRef]
3. Mengfei Chen, He Zhang, Jie Wu, Liang Xu, Di Xu, Jingnan Sun, Yixin He, Xin Zhou, Zhaojing Wang, Lifang Wu, Shaokun Xu, Jinsong Wang, Shu Jiang, Xiangjun Zhou, Andrew R. Hoffman, Xiang Hu, Jifan Hu, Tao Li. 2012. Promotion of the induction of cell pluripotency through metabolic remodeling by thyroid hormone triiodothyronine-activated PI3K/AKT signal pathway. *Biomaterials* **33**:22, 5514-5523. [CrossRef]
4. Nathaniel M. Vacanti, Christian M. Metallo. 2012. Exploring metabolic pathways that contribute to the stem cell phenotype. *Biochimica et Biophysica Acta (BBA) - General Subjects* . [CrossRef]
5. Mika Pietilä , Sami Palomäki , Siri Lehtonen , Ilja Ritamo , Leena Valmu , Johanna Nystedt , Saara Laitinen , Hannnu-Ville Leskelä , Raija Sormunen , Juha Pesälä , Katrina Nordström , Ari Vepsäläinen , Petri Lehenkari . 2012. Mitochondrial Function and Energy Metabolism in Umbilical Cord Blood- and Bone Marrow-Derived Mesenchymal Stem Cells. *Stem Cells and Development* **21**:4, 575-588. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
6. Emily Brookes, Inês de Santiago, Daniel Hebenstreit, Kelly J. Morris, Tom Carroll, Sheila Q. Xie, Julie K. Stock, Martin Heidemann, Dirk Eick, Naohito Nozaki, Hiroshi Kimura, Jiannis Ragoussis, Sarah A. Teichmann, Ana Pombo. 2012. Polycomb Associates Genome-wide with a Specific RNA Polymerase II Variant, and Regulates Metabolic Genes in ESCs. *Cell Stem Cell* **10**:2, 157-170. [CrossRef]
7. David G. Buschke, Jayne M. Squirrell, Jimmy J. Fong, Kevin W. Eliceiri, Brenda M. Ogle. 2012. Cell death, non-invasively assessed by intrinsic fluorescence intensity of NADH, is a predictive indicator of functional differentiation of embryonic stem cells. *Biology of the Cell* n/a-n/a. [CrossRef]
8. Manju Padmasekar , Fatemeh Sharifpanah , Andreas Finkensieper , Maria Wartenberg , Heinrich Sauer . 2011. Stimulation of Cardiomyogenesis of Embryonic Stem Cells by Nitric Oxide Downstream of AMP-Activated Protein Kinase and mTOR Signaling Pathways. *Stem Cells and Development* **20**:12, 2163-2175. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links] [Supplemental material]
9. Clifford DL Folmes, Timothy J Nelson, Andre Terzic. 2011. Energy metabolism in nuclear reprogramming. *Biomarkers in Medicine* **5**:6, 715-729. [CrossRef]
10. Athanasia D Panopoulos, Oscar Yanes, Sergio Ruiz, Yasuyuki S Kida, Dinh Diep, Ralf Tautenhahn, Aída Herrerías, Erika M Batchelder, Nongluk Plongthongkum, Margaret Lutz, W Travis Berggren, Kun Zhang, Ronald M Evans, Gary Siuzdak, Juan Carlos Izpisua Belmonte. 2011. The metabolome of induced pluripotent stem cells reveals metabolic changes occurring in somatic cell reprogramming. *Cell Research* . [CrossRef]
11. Chien-Tsun Chen, Shu-Han Hsu, Yau-Huei Wei. 2011. Mitochondrial bioenergetic function and metabolic plasticity in stem cell differentiation and cellular reprogramming. *Biochimica et Biophysica Acta (BBA) - General Subjects* . [CrossRef]
12. Clifford D.L. Folmes, Timothy J. Nelson, Almudena Martinez-Fernandez, D. Kent Arrell, Jelena Zlatkovic Lindor, Petras P. Dzeja, Yasuhiro Ikeda, Carmen Perez-Terzic, Andre Terzic. 2011. Somatic Oxidative Bioenergetics Transitions into Pluripotency-Dependent Glycolysis to Facilitate Nuclear Reprogramming. *Cell Metabolism* **14**:2, 264-271. [CrossRef]
13. Murat Kasap, Erdal Karaoz, Gurler Akpinar, Ayca Aksoy, Gulay Erman. 2011. A unique Golgi apparatus distribution may be a marker for osteogenic differentiation of hDP-MSCs. *Cell Biochemistry and Function* n/a-n/a. [CrossRef]
14. Melissa A. Kinney , Carolyn Y. Sargent , Todd C. McDevitt . The Multiparametric Effects of Hydrodynamic Environments on Stem Cell Culture. *Tissue Engineering Part B: Reviews*, ahead of print. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
15. Sudip Mandal, Anne G. Lindgren, Anand S. Srivastava, Amander T. Clark, Utpal Banerjee. 2011. Mitochondrial Function Controls Proliferation and Early Differentiation Potential of Embryonic Stem Cells. *STEM CELLS* **29**:3, 486-495. [CrossRef]
16. E. Kenneth Parkinson. 2010. Senescence as a modulator of oral squamous cell carcinoma development. *Oral Oncology* **46**:12, 840-853. [CrossRef]
17. Saiyong Zhu, Wenlin Li, Hongyan Zhou, Wanguo Wei, Rajesh Ambasudhan, Tongxiang Lin, Janghwan Kim, Kang Zhang, Sheng Ding. 2010. Reprogramming of Human Primary Somatic Cells by OCT4 and Chemical Compounds. *Cell Stem Cell* **7**:6, 651-655. [CrossRef]

18. M. E. Phelps, J. R. Barrio. 2010. Correlation of brain amyloid with "aerobic glycolysis": A question of assumptions?. *Proceedings of the National Academy of Sciences* **107**:41, 17459-17460. [CrossRef]
19. Aiwu Cheng, Yan Hou, Mark P Mattson. 2010. Mitochondria and neuroplasticity. *ASN NEURO* **2**:5, 243-256. [CrossRef]
20. Sheller Zabihi, Mary R. Loeken. 2010. Understanding diabetic teratogenesis: Where are we now and where are we going?. *Birth Defects Research Part A: Clinical and Molecular Teratology* **88**:10, 779-790. [CrossRef]
21. Mitsuyo Machida, Yohtaroh Takagaki, Rumiko Matsuoka, Nanako Kawaguchi. 2010. Proteomic comparison of spherical aggregates and adherent cells of cardiac stem cells. *International Journal of Cardiology* . [CrossRef]
22. J. C. St. John, J. Facucho-Oliveira, Y. Jiang, R. Kelly, R. Salah. 2010. Mitochondrial DNA transmission, replication and inheritance: a journey from the gamete through the embryo and into offspring and embryonic stem cells. *Human Reproduction Update* **16**:5, 488-509. [CrossRef]
23. Mika Pietilä , Siri Lehtonen , Marko Närhi , Ilmo E. Hassinen , Hannu-Ville Leskelä , Kari Aranko , Katrina Nordström , Ari Vepsäläinen , Petri Lehenkari . 2010. Mitochondrial Function Determines the Viability and Osteogenic Potency of Human Mesenchymal Stem Cells. *Tissue Engineering Part C: Methods* **16**:3, 435-445. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
24. Alessandro Prigione, Beatrix Fauler, Rudi Lurz, Hans Lehrach, James Adjaye. 2010. The Senescence-Related Mitochondrial/ Oxidative Stress Pathway is Repressed in Human Induced Pluripotent Stem Cells. *STEM CELLS* **28**:4, 721-733. [CrossRef]
25. Susan Chung, D. Kent Arrell, Randolph S. Faustino, Andre Terzic, Petras P. Dzeja. 2010. Glycolytic network restructuring integral to the energetics of embryonic stem cell cardiac differentiation. *Journal of Molecular and Cellular Cardiology* **48**:4, 725-734. [CrossRef]
26. Chien-Tsun Chen, Shu-Han Hsu, Yau-Huei Wei. 2010. Upregulation of mitochondrial function and antioxidant defense in the differentiation of stem cells. *Biochimica et Biophysica Acta (BBA) - General Subjects* **1800**:3, 257-263. [CrossRef]
27. Jinsuk Kang, Arvind Shakya, Dean Tantin. 2009. Stem cells, stress, metabolism and cancer: a drama in two Octs. *Trends in Biochemical Sciences* **34**:10, 491-499. [CrossRef]
28. D. A. Tennant, R. V. Duran, H. Boulahbel, E. Gottlieb. 2009. Metabolic transformation in cancer. *Carcinogenesis* **30**:8, 1269-1280. [CrossRef]
29. J. M. Facucho-Oliveira, J. C. St. John. 2009. The Relationship Between Pluripotency and Mitochondrial DNA Proliferation During Early Embryo Development and Embryonic Stem Cell Differentiation. *Stem Cell Reviews and Reports* **5**:2, 140-158. [CrossRef]
30. M. G. Vander Heiden, L. C. Cantley, C. B. Thompson. 2009. Understanding the Warburg Effect: The Metabolic Requirements of Cell Proliferation. *Science* **324**:5930, 1029-1033. [CrossRef]
31. A. Artero-Castro, H. Kondoh, P.J. Fernández-Marcos, M. Serrano, S. Ramón y Cajal, M.E. LLeonart. 2009. Rplp1 bypasses replicative senescence and contributes to transformation. *Experimental Cell Research* **315**:8, 1372-1383. [CrossRef]
32. Jieru E Lin, Peng Li, Giovanni M Pitari, Stephanie Schulz, Scott A Waldman. 2009. Guanylyl cyclase C in colorectal cancer: susceptibility gene and potential therapeutic target. *Future Oncology* **5**:4, 509-522. [CrossRef]
33. Narasimman Gurusamy , Subhendu Mukherjee , Istvan Lekli , Claudia Bearzi , Silvana Bardelli , Dipak K. Das . 2009. Inhibition of Ref-1 Stimulates the Production of Reactive Oxygen Species and Induces Differentiation in Adult Cardiac Stem Cells. *Antioxidants & Redox Signaling* **11**:3, 589-599. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
34. Arvind Shakya, Robert Cooksey, James E. Cox, Victoria Wang, Donald A. McClain, Dean Tantin. 2009. Oct1 loss of function induces a coordinate metabolic shift that opposes tumorigenicity. *Nature Cell Biology* **11**:3, 320-327. [CrossRef]
35. Hiroshi Kondoh. 2009. Glycolysis during ageing. *Nippon Ronen Igakkai Zasshi. Japanese Journal of Geriatrics* **46**:5, 405-408. [CrossRef]
36. Hiroshi Kondoh. 2008. Cellular life span and the Warburg effect. *Experimental Cell Research* **314**:9, 1923-1928. [CrossRef]
37. Chien-Tsun Chen, Yu-Ru V. Shih, Tom K. Kuo, Oscar K. Lee, Yau-Huei Wei. 2008. Coordinated Changes of Mitochondrial Biogenesis and Antioxidant Enzymes During Osteogenic Differentiation of Human Mesenchymal Stem Cells. *Stem Cells* **26**:4, 960-968. [CrossRef]
38. Dr. Eiichi Araki , Jun-Ichi Miyazaki . 2007. Metabolic Disorders in Diabetes Mellitus: Impact of Mitochondrial Function and Oxidative Stress on Diabetes and Its Complications. *Antioxidants & Redox Signaling* **9**:3, 289-291. [Citation] [Full Text PDF] [Full Text PDF with Links]