Ubiquitous Expression of the Forkhead Box M1B Transgene Accelerates Proliferation of Distinct Pulmonary Cell Types following Lung Injury*

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The delayed early transcription factor Forkhead Box M1B (FoxM1B) is expressed in proliferating cells, but its expression is extinguished in cells undergoing terminal differentiation. Liver regeneration studies with genetically altered mice that either prematurely expressed FoxM1B in hepatocytes or contained a hepatocyte-specific deletion of the Foxm1b allele demonstrated that FoxM1B is critical for regulating the expression of cell cycle genes required for hepatocyte proliferation. Furthermore, preventing the decline in hepatocyte FoxM1B levels during aging was sufficient to increase regenerating hepatocyte proliferation and expression of cell cycle genes to levels found in young regenerating mouse liver. Although these liver regeneration studies demonstrated that FoxM1B is required for hepatocyte proliferation, whether FoxM1B regulates proliferation of cell types other than hepatocytes remains to be determined.

Here, we developed a new TG mouse line in which the −800-base pair Rosa26 promoter was used to drive expression of the FoxM1B transgene in all mouse tissues and found that Rosa26-FoxM1B TG mice were healthy, displaying no developmental defects. We used butylated hydroxytoluene (BHT) lung injury to demonstrate that premature expression of the FoxM1B transgenic protein accelerated proliferation of different lung cell types, including alveolar type II epithelial cells, bronchial epithelial and smooth muscle cells, and endothelial cells of pulmonary capillaries and arteries. This was associated with the earlier expression of the cell cycle promoting cyclin A2, cyclin E, cyclin B1, cyclin F, and cyclin-dependent kinase-1 (Cdk1) genes and diminished protein levels of Cdk inhibitor p21Cip1. Taken together, these results suggest that increasing FoxM1B levels is an effective means to stimulate cellular proliferation during aging and in lung diseases such as emphysema.

The Forkhead Box (Fox) transcription factors are an extensive family of transcription factors consisting of >50 mammalian proteins (1) that share homology in the winged helix DNA binding domain (2, 3). Its members play important roles in regulating transcription of genes involved in cellular proliferation (4–8), differentiation (9–12), and metabolic homeostasis (13, 14). Forkhead Box M1B (FoxM1B) transcription factor (previously known as HFH-11B, Trident, Win, or MPP2) is expressed in proliferating cells, but its expression is extinguished in cells undergoing terminal differentiation (5, 15–17).

In regenerating liver, FoxM1B expression is induced prior to hepatocyte DNA replication (S-phase), and its levels remain elevated throughout the period of proliferation, suggesting that it plays a critical role in regulating hepatocyte proliferation (5). Premature expression of FoxM1B in regenerating transgenic (TG) hepatocytes causes an 8-h acceleration of hepatocyte entry into S-phase and mitosis by stimulating earlier expression of cell cycle genes (4, 6). Liver regeneration studies with 12-month-old (old-aged) TG mice demonstrated that maintaining hepatocyte expression of FoxM1B increased regenerating hepatocyte proliferation and reestablished the expression of cell cycle regulatory genes to levels found in young regenerating mouse liver (7). Likewise, deletion of the Foxm1b floxed (loxP targeted) allele using the Alb-Cre transgene resulted in a significant reduction in regenerating hepatocyte DNA replication and mitosis (8). Reduced hepatocyte DNA replication was associated with increased levels of the cyclin-dependent kinase (Cdk) inhibitor p21Cip1 (p21) and diminished expression of Cdc25A phosphatase, leading to decreased Cdk2 activation and progression into S-phase (8). The decrease in hepatocyte mitosis was associated with diminished expression of the Cdc25A phosphatase and delayed accumulation of cyclin B1, which is required for cyclin B-Cdk1 kinase activation and entry into mitosis (8). Therefore, diminished hepatocyte proliferation in regenerating Alb-Cre Foxm1b−/− liver was associated with altered expression of proteins that limit the Cdk1 and Cdk2 activity required for normal cell cycle progression into DNA replication and mitosis. These studies indicated that FoxM1B is required for cell cycle progression and that increased expression of FoxM1B in old-aged mice is sufficient to reestablish proliferation of regenerating hepatocytes.

Butylated hydroxytoluene (3,5-di-tert-butyl-4-hydroxytoluene; BHT) is a phenolic antioxidant, of which a single administration causes extensive damage to peripheral lung epithelial cells with a substantial influx of inflammatory cells and subsequent pulmonary epithelial cell proliferation 2–4 days after injury.

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‡ The abbreviations used are: FoxM1B, Forkhead Box M1B; WT, wild type; TG, transgenic; Rosa26-FoxM1B TG mice, −800-bp Rosa26 promoter human FoxM1B cDNA transgenic mice; BHT, butylated hydroxytoluene; Cdk, cyclin-dependent kinase; TRA, transthyretin; p21Cip1, Cdk inhibitor; RNA protection assay; BrdUrd, 5-bromo-2′-deoxyuridine; SPB, surfactant protein B; FITC, fluorescein isothiocyanate.

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(16, 19). The initial alveolar epithelial cell injury and repair is followed by endothelial and smooth muscle cell damage and subsequent proliferation 3–5 days after lung injury (18, 19). Previous studies demonstrated that the cellular repair process following BHT lung injury is associated with a transient induction of pulmonary FoxM1B mRNA levels 2–6 days after treatment (20). In this study, we developed a new TG mouse line in which the −800-bp base pair Rosa26 promoter was used to drive ubiquitous expression of the FoxM1B transgene to examine whether premature FoxM1B expression in all pulmonary cell types would accelerate the onset of proliferation following lung injury. We showed that premature expression of FoxM1B transgene protein following BHT lung injury is capable of accelerating proliferation of cells derived from different embryonic cell lineages, including pulmonary epithelial, endothelial, and smooth muscle cells. This stimulated proliferation was associated with the earlier increased expression of the cell cycle genes required for progression into DNA replication and mitosis. Taken together, our studies demonstrate that premature expression of FoxM1B accelerated proliferation of different cell lineages following lung injury, providing a unique mouse model to examine whether maintaining FoxM1B levels in all cell types will prevent the decline in cellular proliferation during aging.

MATERIALS AND METHODS

Rosa26-FoxM1B Mice and BHT Lung Injury—The −800-bp Rosa26 promoter region (a gift from Eric P. Sandgren, University of Wisconsin, Madison) has been reported to drive ubiquitous tissue expression of the green fluorescent protein (GFP) in TG mice (21). To ubiquitously express the FoxM1B transgene, we fused the −800-bp Rosa26 promoter region to the thymosin-β4 (TTR) minigene construct containing the 27-kb human FoxM1B cDNA inserted into the second exon of the TTR minigene that was adjacent to the SV40 virus transcriptional termination sequences (4, 22–25). The initiating methionine ATG codon in the TTR first exon has been mutated, and translation of the FoxM1B protein therefore initiates with the AUG from the FoxM1B cDNA. This human FoxM1B cDNA lacks 800 nucleotides from the mRNA 3′-end, and this deletion stabilizes the FoxM1B transgene mRNA in quiescent cells as described previously (4). We generated eight inbred FVB/N Rosa26-FoxM1B TG mouse lines as verified by PCR screening of mouse tail genomic DNA with TTR minigene-specific primers as described previously (4). Total RNA was prepared from different TG mouse organs using RNA-STAT-60 reagent following procedures recommended by the manufacturer (Tel-Test, “B” Inc., Friendswood, TX). To measure mRNA levels of the FoxM1B transgene in different TG mouse tissues, RNase protection assays (RPAs) were performed with [32P]UTP-labeled antisense RNA synthesized from the mouse FoxM1B exon4 genomic clone (6), the TTR transgene (4), and mCYC-1, mCYC-2, and mCC-1 templates (Pharmingen) as described previously (6–8, 28). Quantitation of expression levels was determined from PhosphorImager scans using the ImageQuant program (Amersham Biosciences). The ribosomal protein L32 or cyclophilin hybridization signals were used for normalization control between different RNA samples.

RESULTS

Generation of Rosa26 Promoter-FoxM1B Transgenic Mice—Our previous studies with TTR-FoxM1B TG mice showed that the FoxM1B transgene protein remained cytoplasmic in quiescent hepatocytes and, therefore, was unable to stimulate them to proliferation (4). However, following either partial hepatectomy or carbon tetrachloride (CCl4) liver injury, the FoxM1B transgene protein translocates to the nucleus earlier and caused an 8-hour acceleration in the onset of hepatocyte DNA replication and mitosis (4, 6). Because FoxM1B is expressed in all proliferating cells (5, 15–17), we wanted to test the hypothesis that premature expression of FoxM1B is sufficient to accelerate proliferation of multiple pulmonary cell types following BHT lung injury. The −800-bp Rosa26 promoter region has been reported to drive ubiquitous tissue expression of the green fluorescent protein in TG mice (21). To express FoxM1B in all mouse tissues, we fused the −800 bp Rosa26 promoter region (21) to the TTR minigene construct (4, 22–25) containing the 3′-truncated human 2.7-kb FoxM1B cDNA (Fig. 1A). We generated eight inbred Rosa26-FoxM1B FVB/N TG mouse lines as verified by PCR screening of mouse tail genomic DNA with TTR minigene-specific primers (4). Consistent with cytoplasmic retention of the FoxM1B transgene protein in quiescent cells (4), none of the Rosa26-FoxM1B TG mice showed abnormal proliferation rates, nor did ectopic expression of the FoxM1B transgene protein influence development of any of TG organs examined (data not shown). RPAs with total RNA isolated from adult WT mouse tissues demonstrated that Foxm1b mRNA is abundantly expressed in thymus and testis, which contain a large population of proliferative cells (Fig. 1B). Lower levels of Foxm1b mRNA were found in spleen, lung, kidney, intestine, and ovaries, which possess lower numbers of dividing cells (Fig. 1B). Our previous studies demonstrated that quiescent adult TG liver exhibited stable expression of the FoxM1B transgene mRNA, which deleted 800 nucleotides of the 3′-untranslated terminal sequences, suggesting that they mediated FoxM1B mRNA degradation in non-dividing cells (4). Because this 3′-end deletion stabilized the FoxM1B transgene mRNA in quies-
cent cells, this enabled us to determine a transgene mRNA expression pattern in TG mouse tissues by using an RPA with the TTR minigene probe (Fig. 1A). The RPA demonstrated that none of the WT tissue RNAs produced an RNase-resistant hybridization product of 310 nucleotides with the TTR transgene probe, which is indicative of FoxM1B transgene expression (Fig. 1C). Rosa26 FoxM1B TG mouse line number 10 exhibited high levels of the FoxM1B transgene in lung, liver, brain, thymus, heart, spleen, kidney, intestine, muscle, and testis and displayed lower levels in skin.

Fig. 1. The ~800-bp Rosa26 promoter directs FoxM1B cDNA expression in TG mice. A, diagrammatically shown is the ~800-bp mouse Rosa26 promoter driving expression of the FoxM1B cDNA that is placed within the TTR minigene construct (4, 22–25). TG mice were created with the Rosa26 promoter region (solid black box) driving expression of the human FoxM1B cDNA (striped box), which was cloned into the TTR second exon that contains the SV40 polyadenylation signal (22, 25). Also depicted on the diagram is the position of the TTR transgene probe. B, FoxM1B is abundantly expressed in adult thymus and testis with lower levels in spleen, lung, kidney, intestine, and ovaries. Total RNA was prepared from different tissues of WT mice and analyzed for mouse FoxM1B and cyclophilin mRNA by RNase protection assay. C, transgene expression in Rosa26 transgenic mice. Total RNA was prepared from different tissues of eight TG mouse lines and analyzed for FoxM1B transgene, mouse TTR, and cyclophilin mRNA by RNase protection assays. Transgenic mouse line 10 exhibited high levels of the FoxM1B transgene in lung, liver, brain, thymus, heart, spleen, kidney, intestine, muscle, and testis and displayed lower levels in skin.

The ubiquitously expressing Rosa26-FoxM1B TG mouse line 10 was selected to examine whether premature FoxM1B levels are sufficient to accelerate proliferation of different pulmonary cell types following BHT lung injury. A single intraperitoneal injection of BHT was given to either Rosa26-FoxM1B TG mice or their WT littermates, and five mice were sacrificed at each of the indicated time points after BHT treatment. Lung tissue was isolated and used for either paraffin embedding or RNA and protein preparation as described under “Materials and Methods.” RPA with BHT-injured lung RNA and the mouse Foxm1b genomic probe allowed us to measure endogenous mouse Foxm1b mRNA levels (6), and the TTR minigene probe was used to measure expression of FoxM1B transgene mRNA (4). Rosa26-FoxM1B TG lungs displayed detectable levels of the FoxM1B transgene mRNA at all time points following BHT lung injury, whereas Foxm1b levels were transiently induced in WT lungs between 36 and 96 h following BHT injury (Fig. 2).

A slight decrease in FoxM1B transgene expression was found after 36 h following BHT injury, suggesting that proliferation may diminish activity of the Rosa26 promoter. Earlier nuclear staining of the FoxM1B transgene protein was detected at 24 h following BHT lung injury (Fig. 3, C and E). In WT lungs, nuclear staining of Foxm1b became detectable at 36 h after BHT injury (Fig. 3D) and reached a maximum by the 42-h time point (Fig. 3F).

To determine whether premature nuclear levels of the FoxM1B transgene protein result in earlier DNA synthesis following BHT lung injury, we monitored pulmonary DNA replication by immunohistochemical staining of BrdUrd incorporation as described previously (4, 26). The Rosa26-FoxM1B TG lungs displayed a statistically significant increase in DNA replication (BrdUrd incorporation) between 32 and 48 h following BHT injury compared with WT littermates at similar time points (Fig. 4A). This is mainly evident at 42 h following BHT injury when the Rosa26-FoxM1B lungs exhibited a 10-fold increase in BrdUrd incorporation compared with WT littermates.
There was no significant difference in BrdUrd incorporation between WT and Rosa26-FoxM1B lungs at 72 h following BHT injury (Fig. 4, D and E). To determine whether earlier DNA replication in Rosa26-FoxM1B TG lungs would cause an increase in mitosis, we detected lung cells undergoing mitosis by immunohistochemical staining with an antibody specific to the phosphorylated form of histone H3 (PH3). These studies revealed an increase in the number of TG pulmonary cells undergoing mitosis between 42 and 48 h following BHT injury compared with WT controls (Fig. 5, A–H). Collectively, these data suggest that premature expression of the FoxM1B transgene caused earlier DNA synthesis and mitosis after BHT lung injury.

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To identify which cell types were stimulated to proliferate earlier in regenerating Rosa26-FoxM1B TG lungs, we used double immunofluorescent staining with a BrdUrd monoclonal antibody along with antibodies specific to a marker protein expressed in a distinct pulmonary cell type.
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lineage. At 42 h following BHT injury, a greater number of double positive BrdUrd and SPB type 2 cells were found in regenerating TG lungs (Fig. 6, A–F; orange arrows) compared with WT lungs (Fig. 6, A–C and G). At 42 h following BHT injury, only Rosa26-FoxM1B TG lungs exhibited double positive BrdUrd and isoelectin B4 alveolar endothelial cells, which are distinguished morphologically from epithelial type II cells by their small nuclei (Fig. 6, D–F and G–L, white arrows). In contrast, endothelial cell proliferation in peripheral WT lungs was not detected until 72 h after BHT injury (Fig. 6, P–S). Furthermore, Rosa26-FoxM1B transgenic lungs displayed earlier BrdUrd staining in peribronchial smooth muscle cells, bronchial epithelial cells, and endothelial cells lining pulmonary arteries at 42 h following BHT injury (Fig. 6, M–O). These results suggest that premature expression of the FoxM1B transgene protein caused earlier proliferation of epithelial, endothelial, and smooth muscle cells after BHT lung injury.

FoxM1B TG Mice Display Earlier Expression of Cyclin Genes and Diminished p21 Levels following Lung Injury—Because FoxM1B regulates the expression of cell cycle regulatory genes in regenerating hepatocytes (4, 6, 8), RPs were performed to examine their temporal expression in regenerating WT and Rosa26-FoxM1B TG lungs following BHT injury. At 24 h post-BHT injury, an earlier expression of S-phase promoting cyclin E and cyclin A2 and M-phase promoting cyclin B1, cyclin F, and Cdk1 genes was found in Rosa26-FoxM1B TG lungs (Fig. 7A). However, by 36 h following BHT injury, similar levels of the same cell cycle genes were found in WT and Rosa26-FoxM1B TG lungs. Western blot analysis demonstrated that expression of Cdk inhibitor p21<sup>Cip1</sup> (p21) protein was diminished in Rosa26-FoxM1B TG lungs between 42 and 48 h post-BHT injury (Fig. 7B). Because p21 expression diminishes S-phase progression by inhibiting Cdk2 activity (29), reduced pulmonary levels of p21 protein may contribute to increased proliferation in BHT-injured TG lungs. Taken together, these data suggest that premature expression of the FoxM1B transgene protein causes earlier increases in cell cycle regulatory pathways that stimulate pulmonary cell proliferation in response to acute lung injury.

**DISCUSSION**

Previous lung and liver regeneration studies demonstrated that increased expression of Foxm1b occurs prior to S-phase and continues throughout the period of proliferation (4–6, 20). Liver regeneration studies demonstrated that premature expression of the FoxM1B transcription factor in TTR-FoxM1B TG hepatocytes causes an 8-h acceleration in the onset of hepatocyte entry into DNA replication and mitosis by stimulating earlier expression of cell cycle genes (4, 6, 6). Likewise, hepatocyte-specific deletion of the Foxm1b <sup>floxed</sup> allele caused a dramatic reduction in hepatocyte DNA replication and mitosis (8). Although these liver regeneration studies established that FoxM1B is required for hepatocyte proliferation, whether FoxM1B regulates proliferation of cell types other than hepatocytes remains to be determined. In this study we created a new TG mouse line in which the −800-bp Rosa26 promoter was used to drive ubiquitous tissue expression of the FoxM1B transgene (Rosa26-FoxM1B TG mice). RNase protection assays demonstrated that Rosa26-FoxM1B TG mice expressed elevated levels of the FoxM1B transgene mRNA in a variety of adult mouse tissues that normally do not express FoxM1B. We used BHT lung injury to determine that premature nuclear expression of FoxM1B accelerated the onset of proliferation of different pulmonary cell types, including alveolar type II epithelial cells, bronchial epithelial and smooth muscle cells, and endothelial cells of pulmonary capillaries and arteries. This is the first demonstration that premature expression of FoxM1B mediates earlier proliferation of pulmonary cells arising from different cellular lineages.

In our current study we demonstrate that premature nuclear localization of the FoxM1B transgene protein caused earlier pulmonary expression of S-phase promoting cyclin E and cyclin A2 following BHT injury. Activation of Cdk2 in complex with either cyclin E or cyclin A2 is required for S-phase progression by phosphorylating the retinoblastoma (RB) protein, releasing RB binding to the E2F transcription factor, and allowing E2F to stimulate expression of genes required for DNA replication (30). We also found diminished protein levels of the Cdk inhibitor p21<sup>Cip1</sup> in regenerating Rosa26-FoxM1B TG lungs, which is consistent with the increased Cdk2 activity required for S-phase progression (28). Furthermore, regenerating TG lungs displayed earlier expression of cyclin B1, cyclin F, and Cdk1 genes, all of which are required for progression into mitosis (31). These data suggest that FoxM1B controls expression of cell cycle regulatory pathways that promote proliferation of different pulmonary cell types in response to acute lung injury. Taken together, these results of this study suggest that increasing FoxM1B levels is an effective means to stimulate proliferation in lung diseases such as emphysema (32–34).

FoxM1B transcription factor is expressed in proliferating cells of embryonic and adult tissues, but its expression is extinguished in cells undergoing terminal differentiation (5, 15, 17). Although FoxM1B expression is induced >40-fold during cellular proliferation (7, 28), its promoter activity is increased only 4-fold in response to serum stimulation (35), suggesting that proliferative signaling also stimulates Foxm1b levels through increased mRNA stability. Consistent with this finding, deletion of the terminal 800 nucleotides from the 3′-end of the human FoxM1B cDNA resulted in stabilization of the FoxM1B transgene mRNA in quiescent mouse hepatocytes (4) and other cell types (this study). Here, we provided evidence that ubiquitous expression of FoxM1B does not perturb mouse embryonic development. This is likely due to the fact that in quiescent cells FoxM1B is tightly regulated by nuclear exclusion through cytoplasmic retention (4). In response to injury-induced proliferative stimuli, the FoxM1B transgene protein translocates into the nucleus and stimulates transcription of cell cycle regulatory genes (4, 6–8, 28).

Recent studies have shown that diminished expression of FoxM1B is associated with age-related reduction in both cellular proliferation and the expression of cell cycle progression genes (7, 36). Liver regeneration studies with 12-month-old (old-aged) TTR-FoxM1B TG mice demonstrate that maintaining hepatocyte expression of FoxM1B increased hepatocyte proliferation to levels found in young regenerating mouse liver (7). Restoration of the young regenerating liver phenotype is associated with increased expression of numerous cell cycle regulatory genes. Furthermore, we used the Alb-Cre recombinase to mediate hepatocyte-specific deletion of the Foxm1b <sup>floxed</sup> allele, and found that Foxm1b deficiency resulted in a significant reduction in regenerating hepatocyte DNA replication and mitosis (8). Diminished hepatocyte proliferation in regenerating Alb-Cre Foxm1b <sup>−/−</sup> liver was associated with the altered expression of proteins that limit the Cdk1 and Cdk2 activity required for normal cell cycle progression into DNA replication and mitosis. These studies indicated that FoxM1B is required for cell cycle progression and that increased expression of FoxM1B in old-aged mice is sufficient to reestablish proliferation of regenerating hepatocytes. However, the TTR-FoxM1B TG mice only maintained expression of the FoxM1B transgene protein in hepatocytes, thus precluding determination of whether sustaining FoxM1B levels in all cell types could prevent age-related decline in cellular proliferation. The develop-
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Fig. 6. Premature expression of FoxM1B causes earlier DNA replication of pulmonary epithelial, endothelial, and smooth muscle cells following BHT injury. Paraffin sections were prepared from lungs of Rosa26-FoxM1B TG and WT mice following BHT lung injury. DNA replication was detected with BrdUrd (BrdU) monoclonal antibody and an anti-mouse antibody conjugated to TRITC (A, D, G, J, and M). Type II epithelial cells were stained with an SPB antibody detected by anti-rabbit antibody conjugated to FITC (B and E), or endothelial cells were visualized using FITC-conjugated isoelectric B4 from Griffiths simplicifolia (lecB4; H and K). A–F, regenerating TG lungs exhibit a greater number of BrdUrd-positive epithelial cells (orange arrows) than regenerating WT lungs. Only regenerating TG lungs exhibited BrdUrd-positive small nuclei, which are likely endothelial cells (white arrows), at 42 h following BHT injury. We show BrdUrd staining for WT (A) and TG (D) lungs, SPB staining for WT (B) and TG (E) lungs, and merging of this staining (C and F). G–L, regenerating TG lungs displayed premature proliferation of endothelial capillary cells (lecB4; white arrows). We show BrdUrd staining for WT (G) and TG (J) lungs, lecB4 endothelial cell staining for WT (H) and TG (K) lungs, and merging of this staining (I and L). M–O, BrdUrd staining of TG lungs depicts earlier proliferation in peribronchiolar smooth muscle cells (M), bronchial epithelial cells (N), and arteriolar endothelial cells (O) at 42 h following BHT injury. P–S, TG lungs display BrdUrd-positive small nuclei at 48 and 72 h following BHT injury (R–S, white arrows), whereas WT lungs show this only at 72 h (Q). Abbreviations are as follows: en, endothelial cells; ep, epithelial cells; sm, smooth muscle cells; Br, bronchle; and Ar, artery. Magnification for A–F and M–S is 400×, and for G–L it is 630×.

Fig. 7. Premature expression of FoxM1B causes an earlier induction of cell cycle regulatory genes following BHT lung injury. Total RNA (A) or protein extracts (B) were prepared from either WT or Rosa26-FoxM1B TG mouse lungs at different hours (Hrs) following BHT injury (indicated by the numbers). A, earlier expression of cell cycle regulatory genes in Rosa26-FoxM1B TG mouse lungs. RNase protection assays were used to analyze for expression of Cdk1 and cyclin mRNA levels with the Pharmingen cyclin RNase protection probes as described previously (6, 7). Arrows indicate the BHT time points at which an early increase in cyclin expression is observed in Rosa26-FoxM1B regenerating lungs. Cyclin levels were normalized to the ribosome large subunit L32 protein mRNA levels, and expression levels are presented with respect to WT lungs prior to BHT injury. B, regenerating Rosa26-FoxM1B transgenic TG mouse lungs exhibit reduced expression of the p21Cip1 protein. Total protein extracts were used for Western blot analysis to measure p21Cip1 protein expression as described previously (8). Protein levels were normalized to β-actin, and expression levels are presented with respect to WT lungs prior to BHT injury.

Activation of Akt is essential for progression into S-phase, because Akt inhibits FoxO4 (AFX/FoxO3a (FKHR-L1)-mediated transcription of the Cdk inhibitor p27Kip1 (p27) (37) and the retinoblastoma-like p130 genes (38). Akt-mediated phosphorylation of the FoxO protein causes nuclear export, thus negatively regulating transcriptional activity of the FoxO proteins (39–41). Unlike the FoxO proteins, whose transcriptional inhibition is required for cell cycle progression, elevated FoxM1B expression is required to stimulate DNA replication in young regenerating liver and lung by diminishing expression of the p21 protein (Ref. 6 and this study). Furthermore, restoring Foxm1b levels in old-aged regenerating liver caused increased hepatocyte proliferation by reducing expression of the p27 protein and stimulating expression of the cell cycle genes required for progression into mitosis (28). Moreover, a significant reduction in hepatocyte DNA replication was found in regenerating Alb-Cre Foxm1b−/− liver, and this reduced proliferation was associated with increased protein expression of the Cdk inhibitor p21Cip1 (p21), which inhibits the S-phase-promoting Cdk2-cyclin E/A complex (8). Similar to the Foxm1b transcription factor, Foxk1−/− mice exhibit a G1 block in skeletal muscle

FoxM1B expression is required to stimulate DNA replication in regenerating liver and lung, leading to life span extension.

招商引资、投资、可再生能源、可再生能源

يفتح FoxM1B الاستجابة المبكرة للتكاثر الجلد بعد إصابة BHT.

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proliferation following injury resulting from a p53-independent increase in p21 protein levels, and skeletal muscle proliferation was restored in Foxk1−/− p21−/− mice (42). However, the phenotype produced in Foxm1b and Foxk1 knockout mice differs in that Foxm1b is also essential for mitosis because it is required for transcription of the M-phase-promoting Cdc25B phosphatase gene (8).

In summary, premature pulmonary expression of the FoxM1B transgene was associated with earlier increases in DNA synthesis and mitosis in regenerating Rosa26-FoxM1B TG lungs. Consistent with the ubiquitous tissue expression pattern of the FoxM1B transgene, earlier proliferation was found in regenerating TG pulmonary epithelial, endothelial, and smooth muscle cells. Premature FoxM1B expression in regenerating TG lungs caused early activation of cell cycle-promoting cyclin A2, cyclin E, cyclin B1, cyclin F, and Cdk1 genes as well as diminished pulmonary levels of cell cycle inhibitor p21Cip1. Taken together, these data suggest that premature FoxM1B expression is sufficient to stimulate cellular proliferation in distinct cell types following lung injury by activating cell cycle regulatory pathways that promote entry into both DNA replication and mitosis.

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