STEM CELLS®

TISSUE-SPECIFIC STEM CELLS

Survivin is Required for Mouse and Human Bone Marrow Mesenchymal Stromal Cell Function

PRATIBHA SINGH[1*], SEIJI FUKUDA[3], LIQIONG LIU[1], BRAHMANANDA REDDY CHITTETI[2] AND LOUIS M. PELUS[1*]

[1]Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, [2] Department of Medicine, Indiana University School of Medicine, Indianapolis, [3]Department of Pediatrics, Shimane University School of Medicine, Izumo, Shimane, Japan

[*]Address correspondence to: Louis M. Pelus, Ph.D., Department of Microbiology and Immunology, Indiana University School of Medicine, 950 West Walnut Street, Indianapolis, IN 46202, Phone: 317-274-7565; Fax: 317-274-7592; E-mail:lpelus@iupui.edu; Pratibha Singh, Ph.D., Department of Microbiology and Immunology, Indiana University School of Medicine, 950 West Walnut Street, Indianapolis, IN 46202, Phone: 317-278-9944; E-mail:pratsing@iupui.edu;

Supported by US Public Health Service grant HL096305 (LMP)

Received October 04, 2016; accepted for publication October 03, 2017; available online without subscription through the open access option.

1066-5099/2017/$30.00/0

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/stem.2727

Key Words. Adult stem cells • Bone marrow stromal cells • Cellular proliferation • Flow cytometry • Mesenchymal stem cells • Adult haematopoietic stem cells

ABSTRACT

Although mesenchymal stromal cells (MSC) have significant potential in cell-based therapies, little is known about the factors that regulate their functions. While exploring regulatory molecules potentially involved in MSC activities, we found that the endogenous multifunctional factor Survivin is essential for MSC survival, expansion, lineage commitment and migration. Pharmacological or genetic blockade of Survivin expression in mouse and human bone marrow MSC enhances caspase 3 and 7 expression and reduces proliferation resulting in fewer MSC and clonogenic CFU-F, whereas ectopic Survivin overexpression in MSC results in their expansion. Survivin is also required for the MSC proliferative responses to basic fibroblast growth factor (b-FGF) and platelet derived growth factor (PDGF). In a wound healing model, Survivin inhibition results in suppression of MSC migration to the wound site. In addition, loss of Survivin in MSCs compromises their hematopoiesis-supporting capacity. These results demonstrate that Survivin is a key regulator of mouse and human MSC function, and suggest that targeted modulation of Survivin in MSCs may have clinical utility to enhance MSC recovery and activity following insult or stress. STEM CELLS *2017; 00:000–000*

SIGNIFICANCE STATEMENT:

Our findings demonstrate that Survivin is essential for the mouse and human MSC survival, proliferation and differentiation. The ability to enhance MSC expansion/survival by growth factor (i.e., b-FGF or PDGF)-mediated Survivin modulation represents a novel therapeutic strategy particularly for hematopoietic regeneration after clinical transplantation, where HSC supportive niche forming MSCs are severely depleted as a consequence of irradiation exposure.

INTRODUCTION

Mesenchymal stromal cells (MSCs) are found throughout adult organisms and are involved in numerous physiological processes, including tissue maintenance and repair (1-3), regulation of hematopoiesis (3) and immunologic responses (4). Pre-clinical and clinical trials using bone marrow (BM) derived MSCs to enhance hematopoietic stem cell transplant and treatment of graft-versus-host disease, spinal cord injury, cartilage and

meniscus repair, and stroke are ongoing; however, MSC engraftment and regenerative potential can be severely limited by their inadequate number, survival, proliferation and multilineage differentiation. Understanding intrinsic and extrinsic factor/mechanism(s) regulating these aspects of MSC biology are required in order to develop new strategies to improve MSC regenerative efficacy.

Survivin is a member of the endogenous inhibitor-of-apoptosis protein (IAP) family. While highly expressed during development and in cancer cells (5, 6), Survivin is also expressed in adult hematopoietic stem and progenitor cells (HSPC) under normal physiological condition and plays an essential role in their function particularly cell proliferation (7, 8). Whether Survivin regulates MSC function however is not known. In this study, we show that both mouse and human BM MSCs expresses Survivin, and furthermore that Survivin is required for MSC survival, proliferation, differentiation and migration.

Methods Summary

C57BL/6 mice were purchased from Harlan Laboratories (Indianapolis, IN). All mouse experiments were approved by the IUSM Institutional Animal Care and Use Committee (IACUC). Human BM cells were purchased from Lonza Biologics (Portsmouth, NH). Detailed procedures for mouse and human cell isolation, MSC staining, purification and expansion, flow cytometry analysis, *in vivo* and *ex vivo* treatments, Survivin gene deletion and overexpression, CFU-F assay and wound healing assay are provided in Supplemental Methods section.

Statistical Analysis: Data are reported as mean ± SEM. Statistical significance was determined by 2-tailed Student's t-test or ANOVA with post-hoc test as appropriate. All data analyzed were normally distributed.

Results and Discussion

Survivin is expressed in mouse and human MSCs and pharmacological or genetic inhibition of Survivin reduces MSC number

We and others have demonstrated that Survivin is required for HSPC function (7-10); however its role in other types of stem cells is largely unknown. Since mesenchymal cells have putative roles in tissue homeostasis and MSCs are crucial components of stem cell niches that support HSPC function (2, 3, 11), we explored whether Survivin plays a role in MSC activity. We first measured intracellular Survivin protein expression in freshly isolated mouse and human bone marrow MSCs identified as $CD45^-Ter119^-CD31^-PDGFR^+CD51^+$ (11) and $CD45^-Ter119^-CD31^-CD271^+PDGFR^{low}$ (12) respectively, which support hematopoietic stem cell function. Flow cytometry analysis revealed that ~ 35% of mouse BM MSC and 31% of adult human BM MSC express Survivin (Figure 1A and Supplementary Figure 1A). Quantitative RT-PCR analysis confirmed Survivin transcripts in mouse and human BM MSCs (Figure 1B). To measure Survivin expression in *ex vivo* expanded MSCs, mouse or human bone marrow $CD45^-Ter119^-CD31^-$ cells were cultured for 2-3 passage and intracellular Survivin protein expression in MSC was measured. Survivin was detected in ~25% of *ex vivo* expended $CD45^-Ter119^-CD31^-PDGFR^+CD51^+$ mouse BM MSCs (Figure 1C) and in ~40% of $CD45^-Ter119^-CD105^+CD90^+PDGFR^{low}$ human BM MSC (Figure 1C and Supplementary Figure 1B). Of note, ~ 90% of total *ex vivo* expanded human bone marrow MSCs were $CD45^-Ter119^-CD31^-lineage^-CD90^+CD105^+PDGFR^{low}$ MSCs and ~ 4.5% of total *ex vivo* expanded mouse MSCs were $CD45^-Ter119^-CD31^-PDGFR^+CD51^+$. Survivin was also detected in the freshly isolated and *ex vivo* expended $CD45^-Ter119^-CD31^-$ population of mouse and human stromal cells, which contains all the sub-types of MSC (Supplementary Figure 1C).

To examine the functional significance of Survivin in MSC, we treated mice *in vivo* with a Survivin inhibitor YM155 that inhibits Survivin expression by blocking its promoter activity (13). Treatment of mice with YM155 (10 mg/kg) for 6 days reduced Survivin protein and mRNA expression in BM MSCs by 2-fold and 6.8-fold respectively (Figure 1D &E). Coincident with inhibition of Survivin expression, the total number of phenotypic BM MSC was decreased by 2.1 fold (Figure 1F) and the total number of BM fibroblast colony forming cells (CFU-F) was reduced by 1.8 fold (Figure 1G).To further validate Survivin function in MSCs, we genetically suppress Survivin in mouse BM MSCs using shRNA. Survivin-specific shRNA reduced Survivin expression by ~ 80% and decreased the ability of MSC to form CFU-F by 3.2 fold (Figure 2A). Conversely, retroviral mediated overexpression of Survivin in mouse MSCs enhanced CFU-F colony formation by 4-fold (Figure 2B).

To explore whether Survivin has a similar role in human MSCs, Survivin gene expression was suppressed using shRNA or with YM155 in *ex vivo* expanded human BM MSCs. Survivin gene deletion in human BM MSCs reduced CFU-F formation by ~6 fold (Figure 2C; left panel) coincident with ~92% reduction in Survivin expression (Figure 2C; right panel). Similarly, treatment of human MSC with 0.1uM YM155 for 5 days reduced total MSC number by 3.2-fold (Figure 2D). These findings suggest that Survivin is important for mouse and human bone marrow MSC maintenance. Consistent with our findings, Suvivin gene deletion in HSPC compartment revealed critical requirement of Survivin in the homeostasis of HSPC (7, 8).

Survivin inhibition enhances MSC apoptosis and decreases their expansion and trilineage differentiation

Survivin has been implicated in antiapoptotic signaling, cell-cycle regulation and cell proliferation (7-10). These

functionally divergent roles of Survivin depend on its subcellular localization, with cytoplasmic Survivin associated with apoptosis inhibition, and nuclear Survivin with cell-cycle (14). To determine whether Survivin is involved in inhibition of MSC apoptosis or regulation of cell proliferation or both, we measured survival and proliferation of mouse MSCs treated with or without YM155. Inhibition of Survivin in *ex vivo* expanded mouse bone marrow stromal cells with YM155 substantially enhanced caspase 3 and 7 expression in $CD45^-Ter119^-CD31^-PDGFR^+CD51^+$ MSC (Supplementary Figure 2A). Survivin inhibition also resulted in significant reduction in MSC proliferation (Supplementary Figure 2B). Similarly, treatment of *ex vivo* expanded human BM MSCs with YM155 enhanced caspases 3 and 7 expression (Figure 3A), while MSC proliferative fraction was suppressed (Figure 3B). These results are in agreement with a dual role of Survivin in both survival and cell proliferation in HSPC. Intracellular topography analysis of MSC Survivin by image stream flow cytometry demonstrated both cytoplasmic and nuclear localization of Survivin supports its role in MSC survival and proliferation (Figure 3C). While Survivin is predominately expressed in the cytoplasm of cancer and leukemia cells and provides resistance against cell death (14, 15). Survivin is differentially expressed in the nucleus at different stages of the cell cycle. Although, only a small percentage of MSCs are in cell cycle, Survivin was detected in all the stages of interphase with maximum expression during G2-M (Figure 3D), which is similar to our previous report of Survivin expression during cell cycle progression in HSPC (9).

Basic fibroblast growth factor (b-FGF) and platelet derived growth factor (PDGF) are cytokines known to expand MSCs (16) and are currently being evaluated for therapeutic MSC expansion. To determine whether Survivin regulates growth factor-mediated MSC expansion, human MSCs were cultured in the presence of b-FGF or PDGF with or without YM155. Both FGF and PDGF substantially enhanced MSC expansion (Figure 3E), which was positively associated with increased Survivin expression (Figure 3F). In contrast, in the presence Survivin inhibitor YM155 both MSC expansion and Survivin expression were reduced. Similarly, b-FGF enhanced mouse $CD45^-Ter119^-CD31^-PDGFR^+CD51^+$ MSC expansion and enhanced Survivin expression, whereas YM155 treatment suppressed Survivin expression and MSC expansion (Supplementary Figure 2C). These data indicate that Survivin is required for basal MSC maintenance as well as for growth factor-mediated MSC expansion.

The hallmark of MSCs is the capacity to differentiate into several mesodermal lineages (17). To investigate whether Survivin regulates MSC differentiation, we examined the effects of Survivin inhibition on the induction of human MSC differentiation into osteocytes, chondrocytes, and adipocytes. Inhibition of Survivin expression in MSCs during lineage differentiation induction reduced the ability of MSCs to differentiate into osteoblasts and chondrocytes and adipocytes (Figure 3G, H &I; Supplementary Figure 3A). The effects of inhibition of Survivin on mouse MSC differentiation were different than human. YM155 treatment reduced mouse MSC differentiation into adipocytes and chondrocytes; however, enhanced osteoblast differentiation (Supplementary Figure 3B). Further study is needed to explore why Survivin differentially regulates mouse and human MSC differentiation.

Survivin regulates MSC migration

Mesenchymal stromal cells have been implicated in tissue regeneration and repair (1, 18, 19) and preclinical studies suggest they enhance the wound healing (20). Since MSC migration/homing to the damaged site is important for tissue repair/healing and Survivin can promote cellular motility (21), we tested whether Survivin regulates MSC trafficking to a wound. Given that 24 hour is sufficient for MSC to reach to the site injury, we measured the effect of Survivin inhibition on MSC migration at 24-hour post YM155 treatment. In an *ex vivo* scratch assay that measures MSC migration into the wounded area, treatment with YM155 for 24 hours reduced the number of MSCs that migrated into the wounded regions by 4 fold (Figure 4A). Similarly, YM155 also reduced mouse MSC migration into the wounded area (Supplementary figure 3C). Interestingly, the total number of viable MSCs was similar in control and YM155 treated cultures (Figure 4B). Since cellular motility is regulated by assembly and disassembly of actin filaments (22), we measured phalloidin staining in YM155 treated MSC, which bind to filamentous actin. Reduced staining of phalloidin in MSC (Figure 4C) suggests that Survivin regulates cellular motility by modulating the cytoskeleton. Since YM155 reduced MSC migration at 24 post treatment, which correlated with phalloidin staining, and given that MSC survival was not reduced at this point, suggest that Survivin regulates MSC motility by affecting actin filament levels and is independent of its role in MSC survival and expansion. In addition, because MSC doubling time is 48 hours and we did not observer significant difference in MSC counts between vehicle and YM155 treated groups at 24 hours post YM155 treatment, further suggests that fewer MSCs in the wounded area was not due an effect on expansion. Given a potential role of Survivin in spindle microtubule assembly that regulates cytokinesis (23) and our new finding that Survivin can contribute to F-actin expression, suggest that Survivin has the ability to regulate multiple components of the cytoskeleton system.

Survivin protects MSC from irradiation and promotes hematopoietic activity

High-dose irradiation used for myeloablation in cancer patients or accidental radiation exposure damages the bone marrow hematopoietic niche components, including MSCs, and limits hematopoietic regeneration (24).

Since our findings suggest that Survivin plays a role in MSC survival and expansion, we examined whether the loss of BM MSCs in response to irradiation exposure is mediated by the downregulation of Survivin expression. Exposure of mice to 650 cGy total body radiation significantly reduced BM MSC Survivin expression (Figure 4D), and was associated with a substantial decrease in phenotypically defined $CD45^{-}Ter119^{-}CD31^{-}PDGFR^{+}CD51^{+}$ MSC and CFU-F counts in BM (Figure 4E &F). Interestingly, treatment of irradiated mice with YM155 further reduced MSC and CFU-F counts. However, treatment of irradiated mice with b-FGF, which enhances Survivin level in MSCs, mitigated the loss of MSC in irradiated mice to levels observed in non-irradiated mice. Conversely, inhibition of Survivin during b-FGF treatment in irradiated mice failed to rescue MSC loss. To investigate whether Survivin can prevent the loss of human MSC in response to irradiation, *ex vivo* expanded human MSCs were exposed to 500 cGy irradiation and treated with YM155 with or without b-FGF for 5 days. Irradiation substantially reduced MSC counts that were further decreased in the presence of the Survivin inhibitor YM155. In contrast, b-FGF partially reverted irradiation mediated MSC loss but failed to rescue YM155 treated MSCs (Figure 4G).

Because MSCs are a crucial constituent of hematopoietic niches and our data suggest a role of Survivin in MSC radioprotection/mitigation, we next tested whether loss of Survivin in MSCs compromises their hematopoiesis-supporting capacity. As expected, deletion of Survivin in non-irradiated human bone marrow MSCs reduced their ability to promote $CD34^{+}$ expansion in a coculture assay (Figure 4H). In addition, irradiated MSCs reduced CD34 expansion compared to non-irradiated MSCs and that was further decreased when Survivin gene was deleted in MSCs. These data suggest that enhancement of Survivin expression in MSCs may protect/rescue them from irradiation induced loss and accelerate hematopoietic regeneration.

Conclusion

Our findings demonstrate that Survivin is essential for the mouse and human MSC survival, proliferation and differentiation. The ability to enhance MSC expansion/survival by growth factor (i.e., b-FGF or PDGF)-mediated Survivin modulation represents a novel therapeutic strategy particularly for hematopoietic regeneration after clinical transplantation, where HSC supportive niche forming MSCs are severely depleted as a consequence of irradiation exposure.

Author Contributions

P.S.: designed the study and executed the experiments, analyzed and interpreted data and wrote the manuscript; S.F.: prepared survivin expressing plasmid and critically read the manuscript; L.L. and B.R.C.: performed RT-PCR and L.M.P.: participated in designing the study, interpreting data, performing the study and wrote the manuscript

References

1 Dimarino AM, Caplan AI, Bonfield TL. Mesenchymal stem cells in tissue repair. FRONT IMMUNOL. 2013;4:201.

2 Festa E, Fretz J, Berry R et al. Adipocyte lineage cells contribute to the skin stem cell niche to drive hair cycling. CELL 2011;146(5):761-771.

3 Mendez-Ferrer S, Michurina TV, Ferraro F et al. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. NATURE 2010;466(7308):829-834.

4 Krampera M, Glennie S, Dyson J et al. Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. BLOOD 2003;101(9):3722-3729.

5 Adida C, Crotty PL, McGrath J et al. Developmentally regulated expression of the novel cancer anti-apoptosis gene survivin in human and mouse differentiation. AM. J. PATHOL. 1998;152(1):43-49.

6 Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. NAT. MED. 1997;3(8):917-921.

7 Fukuda S, Pelus LM. Regulation of the inhibitor-of-apoptosis family member survivin in normal cord blood and bone marrow CD34(+) cells by hematopoietic growth factors: implication of survivin expression in normal hematopoiesis. BLOOD 2001;98(7):2091-2100.

8 Gurbuxani S, Xu Y, Keerthivasan G et al. Differential requirements for survivin in hematopoietic cell development. PROC. NATL. ACAD. SCI. U. S. A 2005;102(32):11480-11485.

9 Fukuda S, Foster RG, Porter SB et al. The antiapoptosis protein survivin is associated with cell cycle entry of normal cord blood CD34(+) cells and modulates cell cycle and proliferation of mouse hematopoietic progenitor cells. BLOOD 2002;100(7):2463-2471.

10 Leung CG, Xu Y, Mularski B et al. Requirements for survivin in terminal differentiation of erythroid cells and maintenance of hematopoietic stem and progenitor cells. J. EXP. MED. 2007;204(7):1603-1611.

11 Pinho S, Lacombe J, Hanoun M et al. PDGFRalpha and CD51 mark human nestin+ sphere-forming mesenchymal stem cells capable of hematopoietic progenitor cell expansion. J. EXP. MED. 2013;210(7):1351-1367.

12 Li H, Ghazanfari R, Zacharaki D et al. Low/negative expression of PDGFR-alpha identifies the candidate primary mesenchymal stromal cells in adult human bone marrow. STEM CELL REPORTS. 2014;3(6):965-974.

13 Nakahara T, Kita A, Yamanaka K et al. YM155, a novel small-molecule survivin suppressant, induces regression of established human hormone-refractory prostate tumor xenografts. CANCER RES. 2007;67(17):8014-8021.

14 Fortugno P, Wall NR, Giodini A et al. Survivin exists in immunochemically distinct subcellular pools and is involved in spindle microtubule function. J. CELL SCI. 2002;115(Pt 3):575-585.

15 Bernardo PS, Reis FR, Maia RC. Imatinib increases apoptosis index through modulation of survivin subcellular localization in the blast phase of CML cells. LEUK. RES. 2012;36(12):1510-1516.

16 Rodrigues M, Griffith LG, Wells A. Growth factor regulation of proliferation and survival of multipotential stromal cells. STEM CELL RES. THER. 2010;1(4):32.

17 Pittenger MF, Mackay AM, Beck SC et al. Multilineage potential of adult human mesenchymal stem cells. SCIENCE 1999;284(5411):143-147.

18 Hyatt AJ, Wang D, van OC et al. Mesenchymal stromal cells integrate and form longitudinally-aligned layers when delivered to injured spinal cord via a novel fibrin scaffold. NEUROSCI. LETT. 2014;569:12-17.

19 Yang Z, Zhu L, Li F et al. Bone marrow stromal cells as a therapeutic treatment for

ischemic stroke. NEUROSCI. BULL. 2014;30(3):524-534.

20 Maxson S, Lopez EA, Yoo D et al. Concise review: role of mesenchymal stem cells in wound repair. STEM CELLS TRANSL. MED. 2012;1(2):142-149.

21 McKenzie JA, Liu T, Goodson AG et al. Survivin enhances motility of melanoma cells by supporting Akt activation and {alpha}5 integrin upregulation. CANCER RES. 2010;70(20):7927-7937.

22 Pollard TD, Borisy GG. Cellular motility driven by assembly and disassembly of actin filaments. CELL 2003;112(4):453-465.

23 Giodini A, Kallio MJ, Wall NR et al. Regulation of microtubule stability and mitotic progression by survivin. CANCER RES. 2002;62(9):2462-2467.

24 Cao X, Wu X, Frassica D et al. Irradiation induces bone injury by damaging bone marrow microenvironment for stem cells. PROC. NATL. ACAD. SCI. U. S. A 2011;108(4):1609-1614.

See www.StemCells.com for supporting information available online. STEM CELLS *; 00:000–000*

Figure1. Survivin is expressed in mouse and human MSCs and pharmacological inhibition of Survivin reduces mouse BM MSCs (A) Left; Representative flow cytometry plots of freshly isolated mouse bone marrow MSCs ($CD45^-Ter119^-CD31^-CD51^+PDGFR\alpha^+$) and human BM MSCs ($CD45^-Ter119^-CD31^-CD271^+PDGFR\alpha^{low}$). Middle: Representative flow plots showing Survivin expression in mouse and human MSCs. Right; Bar graphs showing average Survivin expression in mouse and human MSCs (X±SEM; N= 4 experiments). (B) Survivin transcript quantification in freshly isolated mouse and human BM MSCs by QRT-PCR (X±SEM; N=3 experiments). (C) Survivin expression in *ex vivo* expended mouse and human BM MSCs. Mouse or human BM $CD45^-Ter119^-CD31^-$ cells were cultured in mouse or human specific Mesen-Cult^{TM} media for 2-3 passages and Survivin expression on MSC gated population was determined by flow cytometry (X±SEM; N= 3 experiments). (D-G) Effects of *in vivo* treatment of YM155 on mouse BM MSCs. Mice were injected with YM155 for 6 days (10 mg/kg/day), and (D) intracellular Survivin expression in BM MSC ($CD45^-Ter119^-CD31^-CD51^+PDGFR\alpha^+$) quantitated by flow cytometry. (E) Survivin transcript expression in BM MSCs. (F) MSC counts, and (G) CFU-F counts in BM of control and YM155 treated mice (X±SEM; N=5 mice/group).*, p<0.05 and ***p<0.01.

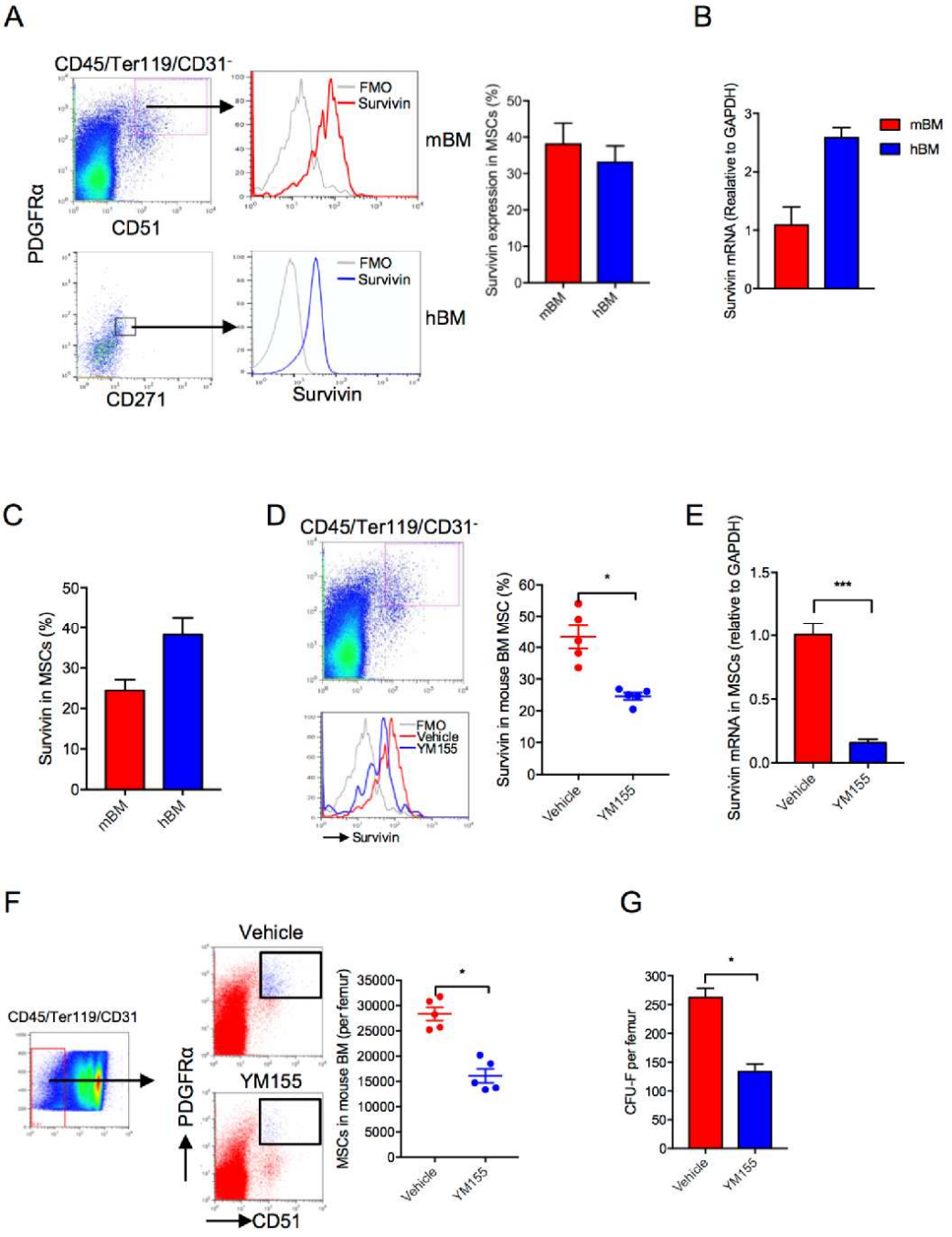


Figure 2. Effects of genetic suppression or overexpression of Survivin on MSCs (A) Survivin gene expression was silenced in FACS sorted BM MSCs ($CD45^-Ter119^-CD31^-CD51^+PDGFR\alpha^+$) using shRNA and clonogenic activity was measured by CFU-F assay (X±SEM; N=3 experiments). (B) Left; representative images showing effect of Survivin overexpression on mouse BM stromal cell CFU-F formation (magnification: 100 X) and Right; average CFU-F counts (X±SEM; N=3 experiments). Survivin was overexpressed in $CD45^-Ter119^-CD31^-$ stromal cells using retroviral transduction method and clonogenic activity of MSCs was measured by CFU-F assay. (C) Left; CFU-F formation from *ex vivo* expanded human BM MSCs transfected with scrambled or Survivin specific shRNA and Right; intracellular Survivin protein expression in MSCs (X±SEM; N=4 experiments). (D) Effect of pharmacological blockade of Survivin on human BM MSCs (X±SEM; N=4 experiments). Human BM MSCs were treated with 0.1µM YM155 for 5 days and MSC recovery was measured by flow cytometry (X±SEM; N=4 experiments).*, p<0.05.

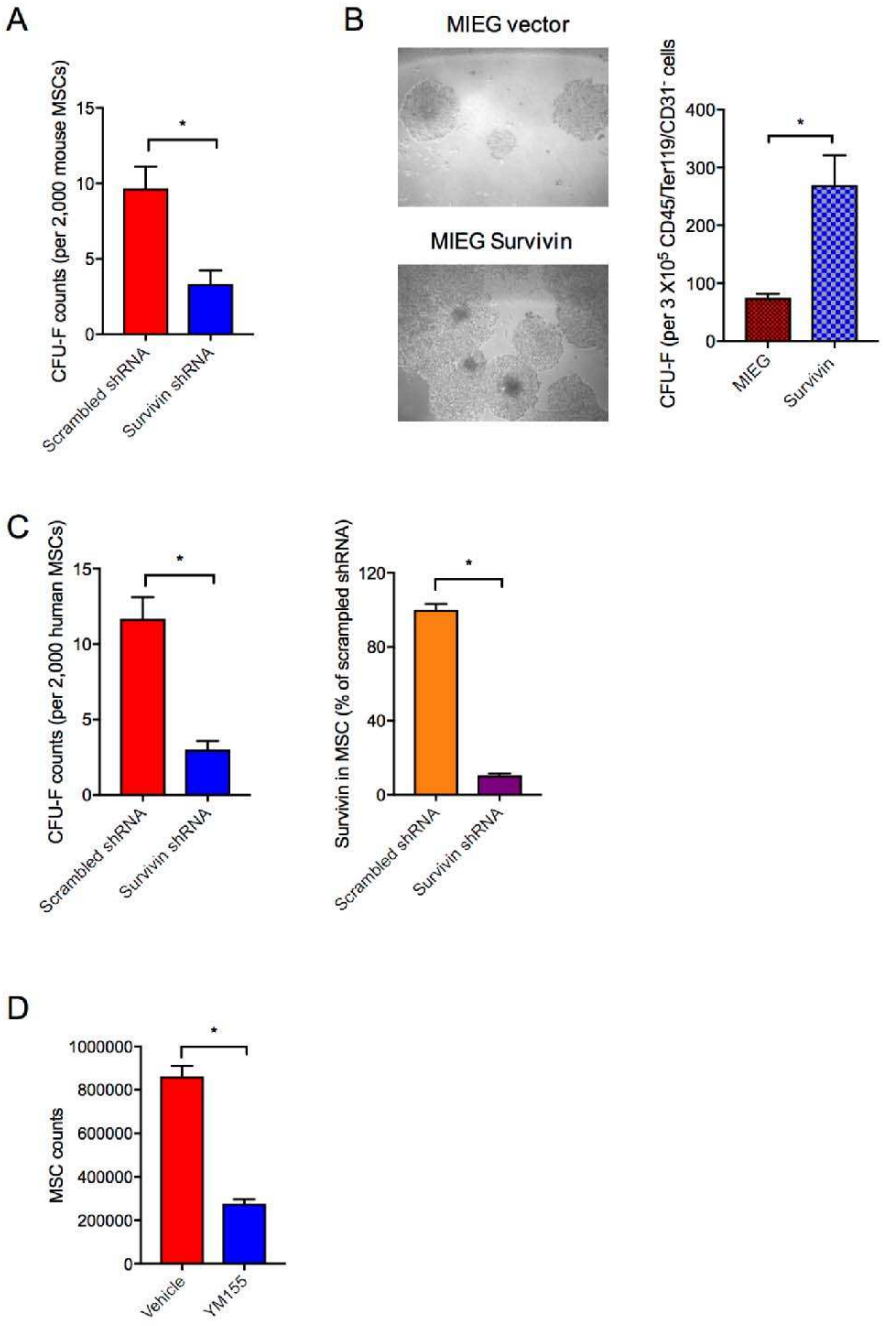
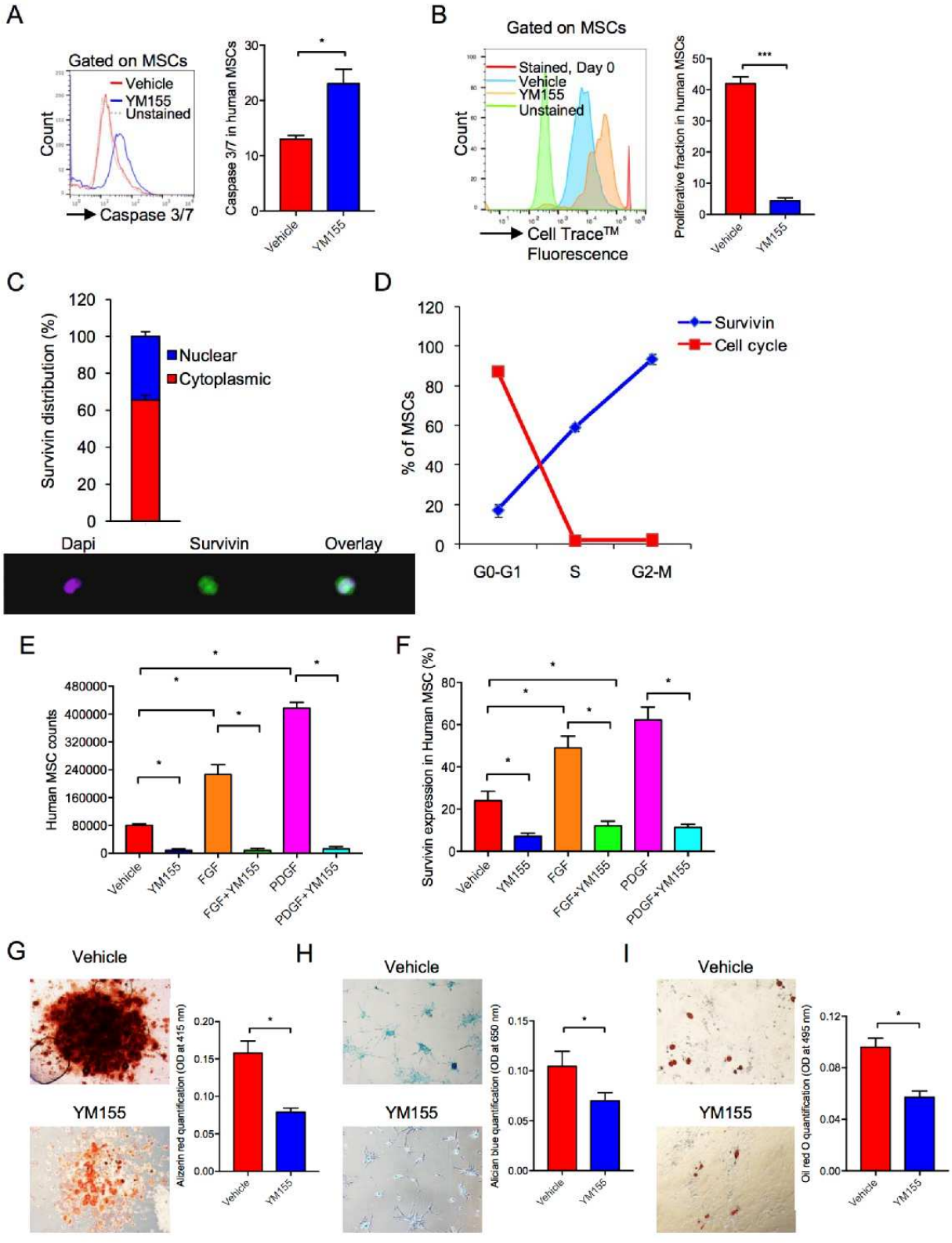


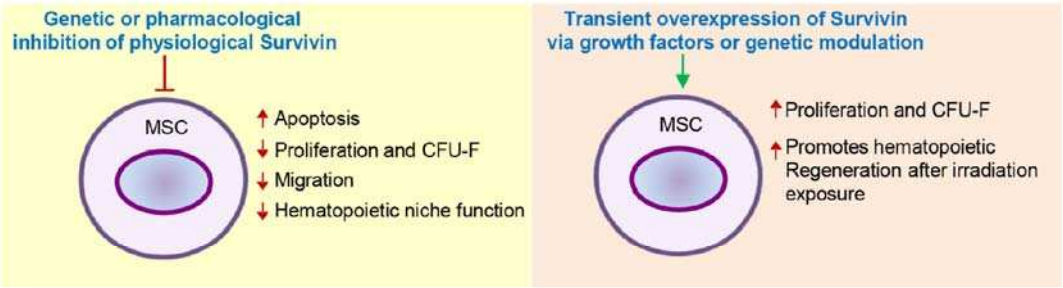
Figure 3. Survivin inhibition induces MSC apoptosis and reduces their proliferation and differentiation (A) Caspase 3/7 expression in YM155 treated and control MSCs. *Ex vivo* expended human MSCs were treated with 0.1μM YM155 for 5 days and caspase 3/7 expression was measured by flow cytotomety (X±SEM; N=4 experiments). (B) MSC proliferation in presence or absence of Survivin inhibitor. *Ex vivo* expanded human BM MSCs were labeled with "Cell Trace^{TM}dye and cultured for 5 days with or without YM155 and dilution of dye measured by flow cytometry (X±SEM; N=4 experiments). (C) Top: The intracellular topography analysis of Survivin expression in *ex vivo* expanded human BM MSCs measured by image stream (X±SEM; N=4 experiments). (C) Bottom: Image depicting nuclear localization of Survivin protein in human MSCs (magnification: 40X). (D) Survivin expression in different stage of cell cycle of human BM MSCs (X±SEM; N=3 experiments). (E) Effect of Survivin blockade on b-FGF and PDGF mediated expansion of human BM MSCs. *Ex vivo* expanded MSCs were cultured in b-FGF (10 ng/ml) or PDGF (20 ng/ml) containing media and treated with YM155 for 5 days and MSCs recovery was determine by flow cytometry analysis. (F) Survivin expression in b-FGF or PDGF expended human MSCs. (X±SEM; N=4 experiments). (G, H&I) Effect of Survivin blockade on human MSC differentiation into osteoblast, chondrocyte and adipocyte. *Ex vivo* expanded human BM MSCs were cultured in osteoblastic, chondrocytic and adipocytic induction media for 15 days with or without YM155. Osteogenic, chondrogenic and adipogenic lineages differentiated cells were stained with Alizarin red, Alcian blue and Oil red O respectively and images were taken at 200X magnification; then, the stains were extracted and measured the absorbance (X±SEM; N=4 experiments).*, $p<0.05$.

Survivin regulates MSC migration and protects MSC from irradiation

(A) Representative images (magnification: 200X) and total counts of MSC migrated into wounded area in the presence or absence of Survivin inhibitor (X±SEM; N=3 experiments). *Ex vivo* expended human MSC monolayers were scraped in a straight line to create a scratch with a p1000 pipet tip. Cell monolayers cultured in MesenCult media with or without YM155. 24 hours later, cells were fixed with 1% PFA and stained with Giemsa to detect the migration of MSCs into wound area (B) Total number of viable MSCs in vehicle or YM155 treated cultures. *Ex vivo* expanded human MSCs were cultured with or without YM155 for 24 hours and cell viability and recovery was determined by live-dead viable dye staining (X±SEM; N=3 experiments). (C) Phallodin staining in human MSCs at 24 hours post YM155 treatment (X±SEM; N=3 experiments). (D, E &F) Survivin protects MSCs from irradiation. Mouse were irradiated at 650 cGy and treated either with YM155 (10 mg/Kg), FGF (100 μg/kg) and YM155 plus FGF for 3 days and BM MSC counts and Survivin were measured by flow cytometry. CFU-F assay was performed to measure their clonal expansion ability (X±SEM; N= 5 mice/ group). (G) Effect of Survivin inhibition on irradiated human MSCs. *Ex vivo* expanded human MSCs were irradiated at 500 cGy, treated with YM155 and FGF alone or in combination and five days post culture MSC recovery was quantitated by flow cytometry (X±SEM; N=3 experiments). (H) Effect of Survivin suppression in human MSCs on their ability to support $CD34^+$ cell expansion. Survivin expression was suppressed in human MSCs using shRNA, irradiated at 500 cGy and cocultured with human bone marrow $CD34^+$ cells. Five days post culture, $CD34^+$ cells expansion was determined by flow cytometry (X±SEM; N=3 experiments). *, $p<0.05$.



Graphical Abstract



Pharmacological or genetic blockade of Survivin expression in mouse and human bone marrow MSC enhances apoptosis and reduces proliferation, CFU-F formation, migration and hematopoietic niche activity, whereas Survivin over expression via growth factors/genetic modulation in MSC results in their expansion and enhancement of hematopoietic regeneration after irradiation injury

