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Perspective

Emerging roles of SIRT1 in vascular endothelial homeostasis

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Sir2 is a NAD$^+$-dependent deacetylase, which regulates life span in multiple model organisms in response to caloric restriction. Mammalian homologues of Sir2 comprise a family of seven proteins termed sirtuins (SIRT1-SIRT7), which have gained considerable attention for their impact on several important physiological processes associated with metabolism and stress resistance. In addition, recent studies point to SIRT1 as a key regulator of vascular endothelial homeostasis controlling angiogenesis, vascular tone and endothelial dysfunction. Here, we review the emerging role of SIRT1 as an important modulator of signaling networks critical for maintaining vascular endothelial homeostasis and discuss SIRT1 as a potential therapeutic target for cardiovascular diseases in the adult.

Histone Deacetylases and Vascular Endothelial Homeostasis

Blood vessels form a highly organized and stereotyped vascular network, which is essential for the delivery of nutrients, gases, macromolecules and cells to all organs in the body. The formation and growth of blood vessels plays a fundamental role for organ growth and regeneration and the vascular network needs to expand significantly during both, embryonic and postnatal development.[1-3] When blood vessel growth is dysregulated, it is a key contributing factor for numerous malignant, ischemic and inflammatory diseases.[1-3] Endothelial cells line the inner surface of the vasculature and are essentially required for angiogenic blood vessel growth, which is the formation of new vessels from pre-existing ones by means of endothelial sprouting, migration and proliferation.[1-3] In addition to their role in angiogenesis, endothelial cells control vascular tone, blood coagulation, and are important mediators of inflammation.[4] Precise control of endothelial cell functions is, thus, critical for the maintenance of blood vessel homeostasis.

The transcriptional regulation of vascular homeostasis requires the coordinated action of several transcription factors and their association with cofactors (coactivators amd corepressors), which allow for a precise time- and signal-dependent regulation of gene expression.[5-7] Histone deacetylases (HDACs) act as critical transcriptional cofactors that are recruited to promoters by sequence-specific transcription factors to regulate gene expression. By removing acetyl groups from nucleosomal histones, HDACs counteract the stimulatory effects of histone acetyltransferases (HATs) resulting in chromatin condensation with consequent transcriptional repression.[8,9] In addition to chromatin remodeling, reversible acetylation has emerged as an important post-translational modification of several non-histone proteins, which are targeted and regulated by HDACs.[10]

HDACs can be classified into three classes based on their sequence homology with yeast proteins Rpd3 (class I), Hda1 (class II) and Sir2 (class III).[11] Class I and II HDACs have been shown to play important roles in vascular biology by controlling endothelial gene expression and vascular development.[12-15] Amongst these enzymes, particularly class II HDACs appear to have a key function for the maintenance of vascular endothelial homeostasis.[12,16] For example, deletion of the HDAC7 gene, which is expressed specifically in endothelial cells during embryonic development, resulted in embryonic lethality due to the loss of vascular integrity in mice.[12] In contrast, the role of members of the structurally and functionally distinct class III enzymes (sirtuins) in vascular endothelial homeostasis remains poorly defined.

The Sirtuin Gene Family

The evolutionary conserved silent information regulator (Sir2) protein is the founding member of class III enzymes, which controls longevity in response to caloric restriction in many organisms, including yeast, worms, flies and possibly mammals.[17-21] In mammals there are 7 homologues of Sir2 termed sirtuins (SIRT1–SIRT7) among which SIRT1 is the closest human homologue of yeast Sir2.[22] Each sirtuin familiy member is characterized by a highly conserved, NAD$^+$-dependent catalytic core domain (sirtuin domain), first identified in the yeast Sir2 protein.[22] Mammalian sirtuins have diverse cellular localizations, modify multiple substrates, and affect numerous cellular functions. Amongst these, SIRT1, SIRT6 and SIRT7 are classified as the nuclear sirtuins, although SIRT1 is not restricted to the nucleus and appears to have important non-nuclear functions.[23] SIRT3, SIRT4 and SIRT5 reside in the mitochondria, whereas SIRT2 is localized predominantly in the cytoplasm.[23] Sirtuins differ not only in their sub-cellular localization, but also in their enzymatic activity. SIRT1, SIRT2, SIRT3 and SIRT5 are NAD-dependent deacetylases, whereas SIRT4 and SIRT6 are primarily mono-ADP-ribosyl transferases with no apparent deacetylase activity on histone substrates in vitro.[23] The enzymatic activity of SIRT7 remains to be determined.

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Sirtuin Functions in Physiology and Disease

While it is not clear whether sirtuins regulate lifespan in mammals, systematic analysis of sirtuin knockout mice has revealed that this group of proteins serves essential functions during embryonic and postnatal development. Amongst the published sirtuin knockout mice, SIRT1- and SIRT6-deficient mice display the most severe phenotypes and are characterized by complex developmental defects.[24-26]

SIRT1 knockout mice are early postnatal lethal and exhibit heart and retinal developmental defects.[24,25] In addition to the essential functions of SIRT1 during embryonic and early postnatal development, SIRT1 maintains its important regulatory role for several homeostatic programs during adulthood. Indeed, SIRT1 has been shown to be a key component in several stress-responsive pathways involved in apoptotic cell death and cellular senescence and to control metabolic processes such as fat and glucose homeostasis.[23,27] The physiologic effects of SIRT1 are mediated by targeting a large number of substrates for deacetylation, including p53, Foxo, PPARγ cofactors NCoR/SMRT, PGC1α or LXR proteins.[28-34] SIRT3-deficient mice, which do not have an obvious phenotype under basal conditions, are characterized by hyperacetylation of mitochondrial proteins suggesting that its main function is to control the acetylation levels of mitochondrial proteins.[35] Analysis of SIRT4 null mice revealed a role of SIRT4 in pancreatic beta cell mitochondria, in which SIRT4 inhibits insulin secretion in response to amino acids, thereby, opposing the effects of caloric restriction in these cells.[36] Although born at Mendelian ratios, SIRT6 deficiency is associated with a severe degenerative syndrome phenotype resembling some aspects of premature ageing leading to death at about 4 weeks of age.[26] Mechanistically, SIRT6 promotes resistance to DNA damage and suppresses genomic instability in association with a role in base excision repair (BER). Consequently, loss of SIRT6 leads to increased genomic instability associated with impaired cellular growth and increased sensitivity to genotoxic stresses.[26] Recently, SIRT7-deficient mice have been shown to undergo a reduction in mean and maximum lifespans and to develop heart hypertrophy and inflammatory cardiomyopathy suggesting roles for this particular sirtuin in stress resistance and apoptosis in cardiomyocytes.[37]

Requirement of Endothelial SIRT1 for Postnatal Vascular Growth

In an effort to identify the functions of sirtuins in vascular endothelial cells, SIRT1 was recently identified as a critical regulator of sprouting angiogenesis during vascular growth.[38] Using a three-dimensional assay of sprouting angiogenesis assay combined with RNA interference to specifically knock down individual SIRT family members as a model system, SIRT1 deacetylase activity was shown to be critical for the angiogenic activity of endothelial cells. Although endothelial cells expressed all sirtuin family members, knock down of SIRT1 was uniquely associated with a near total loss of sprouting angiogenesis in vitro.[38]

The most remarkable function of SIRT1 in the endothelium was revealed by an aberrant neovascularization response of endothelial-restricted SIRT1 mutant mice, in which the deacetylase domain was removed by Cre-mediated excision. Although genetic deletion of SIRT1 activity in the endothelium failed to induce an overt phenotype during embryonic development, analysis of postnatal neovascularization demonstrated that these SIRT1 mutant mice were characterized by an impaired ability to form new vessels in response to angiogenic signals such as ischemic stress.[38] Obviously, the lack of an overt phenotype during embryonic vascular development raises the question whether redundancy among the different sirtuins might compensate for each other's loss in vivo. While this issue needs to be addressed in future studies, these findings also suggest that the signaling pathways, which transduce postnatal angiogenic responses are not necessarily operational during embryonic development and imply that SIRT1 responds to specific signals postnatally to mediate its effects on vascular growth (e.g., upon ischemia). These results are consistent with a role of SIRT1 as a mediator of stress-induced signaling pathways as it has been shown for other cell types and tissues and identify SIRT1 as a signal-responsive regulator of vascular growth.

A clue to the mechanism of how SIRT1 modulates endothelial angiogenic functions came from the time-lapse analysis of segmental vessel formation in transgenic zebrafish embryos with fluorescently labeled endothelial cells in which segmental arteries emanate from the dorsal aorta to form the dorsal longitudinal anastomosing vessel. Compared to the highly organized process of blood vessel formation in the control embryos, SIRT1-deficient zebrafish were characterized by vascular patterning defects and hemorrhages due to dysregulated endothelial sprouting and vessel navigation.[38] Consistent with these observations, loss of SIRT1 activity by either pharmacological inhibition, RNAi-mediated gene silencing or Cre-mediated excision of the floxed SIRT1 deacetylase domain blocked the ability of endothelial cells to form vascular-like sprouts and networks in vitro.[38] Importantly, the reduced angiogenic activity was not a reflection of an overall reduced biological activity as apoptotic cell death or cell cycle arrest were not significantly altered in these cells under basal conditions. Taken together, these findings identify the NAD-dependent deacetylase SIRT1 as critical regulator of endothelial angiogenic functions and postnatal vascular growth. Although the usefulness of SIRT1 as a pharmacological target needs to be addressed in additional ischemia and tumor models, one may speculate that modulation of SIRT1 activity might provide novel opportunities to modulate angiogenesis and blood vessel homeostasis for regeneration and cancer treatment.

Role of SIRT1 in Vascular Tone and Stress-Induced Endothelial Dysfunction

In addition to its function as a regulator of angiogenesis, sirtuins may play a critical role in endothelial homeostasis by regulating the endothelial nitric oxide synthase (eNOS). Endothelial-derived nitric oxide (NO) regulates blood vessel relaxation and provides atheroprotective effects. Resveratrol, a polyphenolic activator of SIRT1, has been shown to increase the expression of eNOS[39] and the combination of resveratrol with the HMG-CoA reductase inhibitors (statins) increased the activation of eNOS resulting in increased functional recovery in a model of acute myocardial infarction.[40] Additionally, chronic resveratrol treatment improved endothelium-dependent relaxation in spontaneous hypertensive rats, however, it did not increase eNOS expression.[41] A recent study by Mattagajashingh and colleagues might explain the mechanism underlying the post-transcriptional activation of eNOS. In this study, SIRT1 has been

shown to promote endothelial-dependent vasodilation by targeting endothelial nitric oxide synthase (eNOS) for deacetylation leading to enhanced nitric oxide production.[42] Likewise, blocking SIRT1 function by transduction of endothelial cells with a deacetylase-defective SIRT1 mutant decreased NO-bioavailability and inhibited endothelium-dependent vasorelaxation. Interestingly, caloric restriction, which is known lo lower blood pressure, led to the deacetylation of eNOS suggesting that the caloric restriction-induced decrease in blood pressure might result from a SIRT1-dependent deacetylation of eNOS.[42] Consistent with an important role of SIRT1 in maintaining endothelial function, a recent report demonstrated that blocking SIRT1 by either pharmacological inhibition or RNAi-mediated knock down induced premature senescence-like phenotypes in endothelial cells.[43] Conversely, overexpression of SIRT1 prevented hydrogen peroxide-induced endothelial senescence suggesting that activating SIRT1 might exert protective effects on the vascular endothelium by preventing stress-induced endothelial dysfunction, an early step in the pathogenesis of several cardiovascular diseases.[43]

In addition to its cell-autonomous role in the vascular endothelial homeostasis, SIRT1 might also modulate vascular homeostasis by beneficially affecting several metabolic pathways involved in cardiovascular disease progression such as cholesterol metabolism, glucose homeostasis and insulin resistance.[27] Recent reports have shown that SIRT1 deacetylates and thereby activates the nuclear receptor LXR, a central transcriptional regulator of reverse cholesterol transport.[34] By modulating the activity of this nuclear factor, SIRT1 might favor cholesterol removal from peripheral tissues and, thus, prevent the formation of age-associated atherosclerotic lesion formation.[34,44]

SIRT1 has also been shown to improve insulin resistance,[45,46] a key contributing factor for the development of type 2 diabetes. Whereas loss of SIRT1 activity induces insulin resistance, overexpression of SIRT1 or enhancing its activity by treatment with resveratrol improved insulin sensitivity especially under insulin-resistant conditions.[45,46] Taken together, these findings advance the knowledge of SIRT1 as a key regulator of tissue homeostasis by defining the vascular endothelium as an important target tissue for the direct and indirect actions of SIRT1.

Molecular Targets of SIRT1 in Endothelial Cells

Besides histones, several non-histone proteins are targeted by SIRT1 for deacetylation. Among these are several transcription factors, transcriptional cofactors and chromatin modifying enzymes, including p53, Foxo, NCoR/SMRT, PGC1α or SUV39H1, which mediate specific SIRT1-dependent cellular responses.[28-33,47]

Several studies highlight the importance of SIRT1 in mediating stress resistance by interfering with stress-responsive pathways (e.g., p53, Foxo or NBS1).[28-31,48-50] For example, SIRT1 has been shown to promote cell survival in response to cellular stress by deacetylating the tumor suppressor protein p53, which downregulates p53 stability and activity.[24,28,29,51] In addition, SIRT1 associates with forkhead transcription factors (Foxo) upon exposure to hydrogen peroxide (H_2O_2) to mediate Foxo deacetylation and target gene expression.[30,48,52] This stress-responsive and evolutionary highly conserved interaction of SIRT1 and Foxo transcription factors has been adopted by the vascular endothelium to control its angiogenic activity. Indeed, Foxo family members have been identified as essential negative regulators of blood vessel formation among which Foxo1 appears to be the physiologically most important repressor of endothelial integrity.[53,54] Using gain- and loss-of-function approaches, it has been demonstrated that SIRT1 has the ability to repress Foxo1-dependent transcriptional activity in endothelial cells[38] and, thus, point to this transcription factor as an effector in the SIRT1-dependent angiogenic signaling pathway. However, the mode of Foxo regulation by SIRT1 remains controversial, with some data suggesting that deacetylation decreases[31,38,55] and others that it increases Foxo activity.[30,48,49,56] The molecular basis for these apparent differences is currently unclear. However, given the fact that Foxo1 is acetylated on several lysine residues,[30,52,57] it is tempting to speculate that dependent on cofactor recruitment of Foxo1, SIRT1 might only have access to a subset of acetylated lysine residues leading to a signal-dependent acetylation pattern of Foxo1, thereby, modulating its biological activity. In addition, it is notable that acetylation of Foxo1 might compete with other posttranslational modifications such as ubiquitylation or sumoylation for the same lysine acceptor sites to regulate gene expression.[58] The transcriptional output might, thus, depend on the combination of distinct posttranslational modifications converging on Foxo lysine residues.

Although Foxo1 is an important deacetylation target of SIRT1 in the vascular endothelium, the data available so far point to the existence of additional molecular targets of SIRT1, as neither the dysregulated gene expression nor the loss of angiogenic sprouting could be fully attributed to an inhibition of Foxo1.[38] Given the multitude of SIRT1 interacting partners described in other cells and tissues,[27] it is more than likely that SIRT1 has additional partners in vascular endothelial cells to mediate its specific effects. Intriguingly, SIRT1 has been shown to interact with eNOS,[42] which plays a key role in maintaining vascular homeostais. SIRT1 has been shown to bind to eNOS and deacetylate lysines 496 and 506 in the calmodulin-binding domain of eNOS leading to enhanced NO production. [42] Given that eNOS-derived NO is not only essential for endothelial-dependent vasorelaxation,[59] but also for endothelial cell survival, migration and postnatal neovascularization,[60-64] NO might contribute to the Foxo-independent effects of the SIRT1-dependent regulation of sprouting angiogenesis. Of note, the Foxo transcription factors Foxo1 and Foxo3a have been shown to repress eNOS expression[53] suggesting a crosstalk between SIRT1, Foxos and eNOS. However, eNOS mRNA was not significantly downregulated in SIRT1-silenced endothelial cells[38] implying that additional cofactors contribute to the Foxo-dependent regulation of eNOS expression.

In overexpression studies, Takata and colleagues reported that SIRT1 associated with the Hairy and Enhancer-of-split basic helix-loop-helix (bHLH) transcriptional repressor Hey2 to mediate transcriptional repression.[65] Hey2 is the human homologue of the zebrafish gridlock, which has been shown to be an important regulator of endothelial gene expression and mutant mice lacking *Hey1* and *Hey2* are embryonically lethal due to defects in vascular development.[66] While it needs to be proven whether such an interaction occurs in endothelial cells, Hey2 appears to be an attractive target, given its essential functions in the cardiovascular system and recent reports demonstrating the importance of the Delta-like 4/Notch/Hey signaling cascade for controlling tip cell formation and vascular patterning.[67-71] Thus, it is indeed tempting to hypothesize that SIRT1 modulates endothelial angiogenic activity by interfering with this pathway, e.g., by associating with Hey2.

It is well conceivable that in addition to the targets outlined here in brief (Fig. 1), SIRT1 has additional binding partners in endothelial cells and, thus, point to this deacetylase as a point of convergence of several signaling pathways critical for homeostatic endothelial functions. In the future, it will be interesting to identify these novel SIRT1-regulated factors and to investigate their involvement in vascular growth and disease.

Gene Targets of SIRT1 in Endothelial Cells

By associating with transcription factors and transcriptional cofactors, SIRT1 acts as an important modulator of gene expression. In endothelial cells, gene targets (direct and indirect) of SIRT1 have been assessed by RNA interference-mediated knock down of SIRT1. This expression screen revealed that loss of SIRT1 activity leads to the dysregulation of several genes with essential roles in cardiovascular development and homeostasis. Among the regulated genes were several transcription factors (e.g., *Fli1*, *Hex*), members of the TGFβ signaling cascade (e.g., *SMAD7*, *Tak1*), cell surface receptors (e.g., *Flt1*, *CXCR4*) and important signaling molecules involved in angiogenesis and vascular remodeling.[38]

Although the upstream mechanisms (direct and indirect) leading to the dysregulation of most of these genes remain to be determined, they provide interesting mechanistic insights of how SIRT1 might coordinate signaling networks and affects endothelial cell behavior. As such, the SIRT1 siRNA induced reduction of MMP14 (MT1-MMP), a membrane-anchored matrix metalloproteinase essential for tip cell activity during sprouting angiogenesis,[72,73] suggests that it might contribute to the path-finding defects observed in the SIRT1-deficient zebrafish.[38]

In addition, the altered expression of genes involved in TGFβ signaling suggests that SIRT1 might be a key modulator of this signaling pathway by interfering with an upstream regulator or even an TGFβ transcriptional effector such as SMAD proteins.[38] Consistent with these considerations, SIRT1 has been reported to interact and deacetylate SMAD7, which is an auto-inhibitory downstream molecule of TGFβ signaling.[74]

A caveat to the identification of SIRT1-modulated genes is that SIRT1 appears to be a context/signal-dependent regulator of cellular responses. While the gene expression profile in SIRT1-deficient endothelial cells clearly support a role of SIRT1 in the transcriptional control of endothelial homeostatic functions, it remains to be determined whether the gene expression changes observed in the microarray analysis performed under basal conditions reflect the entire signaling network of SIRT1 in vascular homeostasis. It is, thus, tempting to speculate that the gene targets of SIRT1 will most likely depend on the activity and acetylation pattern of the signaling pathway targeted by SIRT1.

SIRT1 as a "Drugable" Target for Cardiovascular Disease

The important role of SIRT1 for vascular endothelial homeostasis in vivo as well as its obvious beneficial effects on metabolic pathways, which critically contribute to the progression of cardiovascular disease, suggest opportunities for therapeutically exploiting the function of SIRT1 in the setting of cardiovascular diseases. Because SIRT1 targets several proteins in distinct signaling pathways for deacetylation, modulation of SIRT1 activity could alter the biological activity of entire signaling networks and thereby modify complex disease processes such as pathological angiogenesis or atherosclerosis. Indeed, recent studies reported that resveratrol mimicked the anti-ageing effects of calorie restriction in mice fed a high-fat diet and ameliorated insulin resistance and prolonged survival.[75,76] Although it is still not clear, whether resveratrol acts directly or indirectly through SIRT1 in vivo,[77] the recently developed small molecule activators of SIRT1 that are structurally unrelated to, and 1.000-fold more potent than resveratrol, induce many of the beneficial metabolic changes observed after caloric restriction/resveratrol treatment, point to SIRT1 as a promising new therapeutic approach for treating diseases of ageing.[46] Thus, the identification of an intrinsic function of SIRT1 in the endothelium suggests that diseases of the cardiovascular system might be particularly sensitive to SIRT1-modifiying drugs. Based on these considerations, one would predict that activation of SIRT1 would not only benefically affect established risk factors such type 2 diabetes or cholesterol homeostasis, but would also directly protect the endothelium by preventing endothelial dysfunction. Likewise, SIRT1 activation might ameloriate ischemic vascular diseases by promoting angiogenesis or by enhancing progenitor cell-mediated neovascularization. As such, resveratrol was recently shown to increase the number of endothelial progenitor cells in vitro.[78-80]

The finding that SIRT1 acts as an important regulator of postnatal vascular growth suggests that strategies to inhibit SIRT1 activity could also provide an opportunity for anti-angiogenesis therapies. Dysregulation of angiogenic growth has been shown to contribute to numerous pathologies including malignant or inflammatory disorders and anti-angiogenic agents such as α-VEGF have been shown to prolong the survival of cancer patients.[1,81]

While SIRT1 clearly plays a critical role in development, ageing and protection against cancer development, several tumors exhibit enhanced expression levels of SIRT1 and appear to be dependent

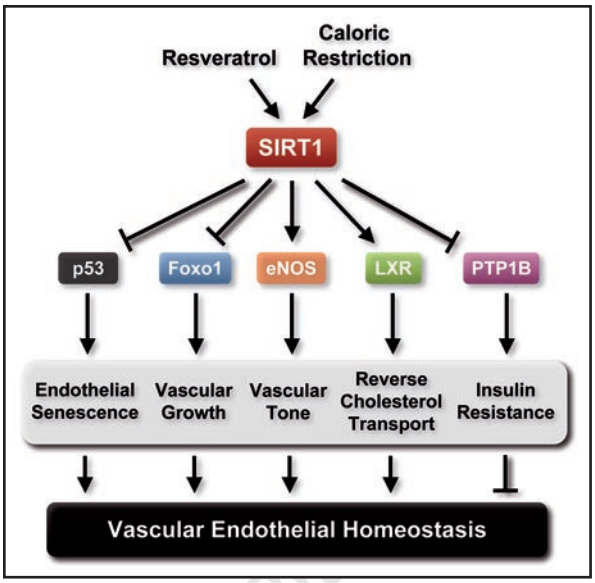

Figure 1. Signaling networks of SIRT1 involved in the maintenance of vascular homeostasis. In endothelial cells SIRT1 modulates the transcriptional activity of Foxo1 and p53 and activates the enzymatic activity of the endothelial nitric oxide synthase (eNOS). SIRT1 regulates cholesterol homeostasis by deacetylating the nuclear factor LXR in hepatocytes. By repressing the expression of the phosphatase PTP1B at the chromatin level, SIRT1 improves insulin sensitivity under insulin resistant conditions.

on SIRT1 for proliferation and survival.[82] Therefore, SIRT1-specific inhibitors may be useful and might evolve as additional chemotherapeutic agents for tumors that depend on SIRT1 activity. Current strategies for anti-angiogenic therapy target early steps in the signal transduction cascade such as ligands or their cognate receptors.[1,81,83] Given the multitude of downstream factors targeted by SIRT1 for deacetylation, SIRT1 might act as a nexus of several pathways common to the growth and vascularization of tumors and might, thus, represent alternative approach for blocking tumor progression.

While these considerations imply that strategies to modulate the activity of SIRT1 might serve as a potential therapy for age-associated cardiovascular diseases or cancer, the rather ubiquitous expression and broad effects of SIRT1 might also pose significant hurdles with regard to specificity and side-effects.

Outlook

In summary, the findings of the recent studies point to SIRT1 as a novel regulator of vascular endothelial homeostasis, which plays a key role in the maintenance of vessel function. However, many questions remain to be answered. For instance, is SIRT1 active in pathologic vessels in growing tumors or other vascular-related diseases? Is SIRT1 activity recruited by specific pathways such as VEGF/VEGFR2 signaling to mediate its cellular responses? Likewise, it will be interesting to investigate, how SIRT1 activity and expression is controlled in several physiologic and pathologic programs. Given the fact that most work so far focused on the biological actions of the closest Sir2 homologue, SIRT1, in the vascular system, further studies will be required to address the functions of the other sirtuin family members in the vasculature and to link their mode of action with specific vascular programs.

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References

1. Carmeliet P. Angiogenesis in health and disease. Nat Med 2003; 9:653-60.
2. Jain RK. Molecular regulation of vessel maturation. Nat Med 2003; 9:685-93.
3. Adams RH, Alitalo K. Molecular regulation of angiogenesis and lymphangiogenesis. Nat Rev Mol Cell Biol 2007; 8:464-78.
4. Libby P, Aikawa M, Jain MK. Vascular endothelium and atherosclerosis. Handb Exp Pharmacol 2006; 285-306.
5. Hamik A, Wang B, Jain MK. Transcriptional regulators of angiogenesis. Arterioscler Thromb Vasc Biol 2006; 26:1936-47.
6. Dejana E, Taddei A, Randi AM. Foxs and Ets in the transcriptional regulation of endothelial cell differentiation and angiogenesis. Biochim Biophys Acta 2007; 1775:298-312.
7. Papanicolaou KN, Izumiya Y, Walsh K. Forkhead transcription factors and cardiovascular biology. Circ Res 2008; 102:16-31.
8. Cheung P, Allis CD, Sassone-Corsi P. Signaling to chromatin through histone modifications. Cell 2000; 103:263-71.
9. Jenuwein T, Allis CD. Translating the histone code. Science 2001; 293:1074-80.
10. Yang XJ. Multisite protein modification and intramolecular signaling. Oncogene 2005; 24:1653-62.
11. Grozinger CM, Schreiber SL. Deacetylase enzymes: biological functions and the use of small-molecule inhibitors. Chem Biol 2002; 9:3-16.
12. Chang S, Young BD, Li S, Qi X, Richardson JA, Olson EN. Histone deacetylase 7 maintains vascular integrity by repressing matrix metalloproteinase 10. Cell 2006; 126:321-34.
13. Kim MS, Kwon HJ, Lee YM, Baek JH, Jang JE, Lee SW, Moon EJ, Kim HS, Lee SK, Chung HY, Kim CW, Kim KW. Histone deacetylases induce angiogenesis by negative regulation of tumor suppressor genes. Nat Med 2001; 7:437-43.
14. Rossig L, Li H, Fisslthaler B, Urbich C, Fleming I, Forstermann U, Zeiher AM, Dimmeler S. Inhibitors of histone deacetylation downregulate the expression of endothelial nitric oxide synthase and compromise endothelial cell function in vasorelaxation and angiogenesis. Circ Res 2002; 91:837-44.
15. Rossig L, Urbich C, Bruhl T, Dernbach E, Heeschen C, Chavakis E, Sasaki K, Aicher D, Diehl F, Seeger F, Potente M, Aicher A, Zanetta L, Dejana E, Zeiher AM, Dimmeler S. Histone deacetylase activity is essential for the expression of HoxA9 and for endothelial commitment of progenitor cells. J Exp Med 2005; 201:1825-35.
16. Mottet D, Bellahcene A, Pirotte S, Waltregny D, Deroanne C, Lamour V, Lidereau R, Castronovo V. Histone deacetylase 7 silencing alters endothelial cell migration, a key step in angiogenesis. Circ Res 2007; 101:1237-46.
17. Imai S, Armstrong CM, Kaeberlein M, Guarente L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature 2000; 403:795-800.
18. Smith JS, Brachmann CB, Celic I, Kenna MA, Muhammad S, Starai VJ, Avalos JL, Escalante-Semerena JC, Grubmeyer C, Wolberger C, Boeke JD. A phylogenetically conserved NAD+-dependent protein deacetylase activity in the Sir2 protein family. Proc Natl Acad Sci USA 2000; 97:6658-63.
19. Landry J, Slama JT, Sternglanz R. Role of NAD(+) in the deacetylase activity of the SIR2-like proteins. Biochem Biophys Res Commun 2000; 278:685-90.
20. Guarente L, Picard F. Calorie restriction—the SIR2 connection. Cell 2005; 120:473-82.
21. Longo VD, Kennedy BK. Sirtuins in aging and age-related disease. Cell 2006; 126:257-68.
22. Frye RA. Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins. Biochem Biophys Res Commun 2000; 273:793-8.
23. Michan S, Sinclair D. Sirtuins in mammals: insights into their biological function. Biochem J 2007; 404:1-13.
24. Cheng HL, Mostoslavsky R, Saito S, Manis JP, Gu Y, Patel P, Bronson R, Appella E, Alt FW, Chua KF. Developmental defects and p53 hyperacetylation in Sir2 homolog (SIRT1)-deficient mice. Proc Natl Acad Sci USA 2003; 100:10794-9.
25. McBurney MW, Yang X, Jardine K, Hixon M, Boekelheide K, Webb JR, Lansdorp PM, Lemieux M. The mammalian SIR2alpha protein has a role in embryogenesis and gametogenesis. Mol Cell Biol 2003; 23:38-54.
26. Mostoslavsky R, Chua KF, Lombard DB, Pang WW, Fischer MR, Gellon L, Liu P, Mostoslavsky G, Franco S, Murphy MM, Mills KD, Patel P, Hsu JT, Hong AL, Ford E, Cheng HL, Kennedy C, Nunez N, Bronson R, Frendewey D, Auerbach W, Valenzuela D, Karow M, Hottiger MO, Hursting S, Barrett JC, Guarente L, Mulligan R, Demple B, Yancopoulos GD, Alt FW. Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. Cell 2006; 124:315-29.
27. Haigis MC, Guarente LP. Mammalian sirtuins—emerging roles in physiology, aging, and calorie restriction. Genes Dev 2006; 20:2913-21.
28. Vaziri H, Dessain SK, Ng Eaton E, Imai SI, Frye RA, Pandita TK, Guarente L, Weinberg RA. hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase. Cell 2001; 107:149-59.
29. Luo J, Nikolaev AY, Imai S, Chen D, Su F, Shiloh A, Guarente L, Gu W. Negative control of p53 by Sir2alpha promotes cell survival under stress. Cell 2001; 107:137-48.
30. Brunet A, Sweeney LB, Sturgill JF, Chua KF, Greer PL, Lin Y, Tran H, Ross SE, Mostoslavsky R, Cohen HY, Hu LS, Cheng HL, Jedrychowski MP, Gygi SP, Sinclair DA, Alt FW, Greenberg ME. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. Science 2004; 303:2011-5.
31. Motta MC, Divecha N, Lemieux M, Kamel C, Chen D, Gu W, Bultsma Y, McBurney M, Guarente L. Mammalian SIRT1 represses forkhead transcription factors. Cell 2004; 116:551-63.
32. Picard F, Kurtev M, Chung N, Topark-Ngarm A, Senawong T, Machado De Oliveira R, Leid M, McBurney MW, Guarente L. Sirt1 promotes fat mobilization in white adipocytes by repressing PPARgamma. Nature 2004; 429:771-6.
33. Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P. Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. Nature 2005; 434:113-8.
34. Li X, Zhang S, Blander G, Tse JG, Krieger M, Guarente L. SIRT1 deacetylates and positively regulates the nuclear receptor LXR. Mol Cell 2007; 28:91-106.
35. Lombard DB, Alt FW, Cheng HL, Bunkenborg J, Streeper RS, Mostoslavsky R, Kim J, Yancopoulos G, Valenzuela D, Murphy A, Yang Y, Chen Y, Hirschey MD, Bronson RT, Haigis M, Guarente LP, Farese RV Jr, Weissman S, Verdin E, Schwer B. Mammalian Sir2 homolog SIRT3 regulates global mitochondrial lysine acetylation. Mol Cell Biol 2007; 27:8807-14.
36. Haigis MC, Mostoslavsky R, Haigis KM, Fahie K, Christodoulou DC, Murphy AJ, Valenzuela DM, Yancopoulos GD, Karow M, Blander G, Wolberger C, Prolla TA, Weindruch R, Alt FW, Guarente L. SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic beta cells. Cell 2006; 126:941-54.
37. Vakhrusheva O, Smolka C, Gajawada P, Kostin S, Boettger T, Kubin T, Braun T, Bober E. Sirt7 Increases Stress Resistance of Cardiomyocytes and Prevents Apoptosis and Inflammatory Cardiomyopathy in Mice. Circ Res 2008.
38. Potente M, Ghaeni L, Baldessari D, Mostoslavsky R, Rossig L, Dequiedt F, Haendeler J, Mione M, Dejana E, Alt FW, Zeiher AM, Dimmeler S. SIRT1 controls endothelial angiogenic functions during vascular growth. Genes Dev 2007; 21:2644-58.
39. Wallerath T, Li H, Godtel-Ambrust U, Schwarz PM, Forstermann U. A blend of polyphenolic compounds explains the stimulatory effect of red wine on human endothelial NO synthase. Nitric Oxide 2005; 12:97-104.
40. Penumathsa SV, Thirunavukkarasu M, Koneru S, Juhasz B, Zhan L, Pant R, Menon VP, Otani H, Maulik N. Statin and resveratrol in combination induces cardioprotection against myocardial infarction in hypercholesterolemic rat. J Mol Cell Cardiol 2007; 42:508-16.
41. Rush JW, Quadrilatero J, Levy AS, Ford RJ. Chronic resveratrol enhances endothelium-dependent relaxation but does not alter eNOS levels in aorta of spontaneously hypertensive rats. Exp Biol Med (Maywood) 2007; 232:814-22.

42. Mattagajasingh I, Kim CS, Naqvi A, Yamamori T, Hoffman TA, Jung SB, DeRicco J, Kasuno K, Irani K. SIRT1 promotes endothelium-dependent vascular relaxation by activating endothelial nitric oxide synthase. Proc Natl Acad Sci USA 2007; 104:14855-60.
43. Ota H, Akishita M, Eto M, Iijima K, Kaneki M, Ouchi Y. Sirt1 modulates premature senescence-like phenotype in human endothelial cells. J Mol Cell Cardiol 2007; 43:571-9.
44. Bordone L, Cohen D, Robinson A, Motta MC, van Veen E, Czopik A, Steele AD, Crowe H, Marmor S, Luo J, Gu W, Guarente L. SIRT1 transgenic mice show phenotypes resembling calorie restriction. Aging Cell 2007; 6:759-67.
45. Sun C, Zhang F, Ge X, Yan T, Chen X, Shi X, Zhai Q. SIRT1 improves insulin sensitivity under insulin-resistant conditions by repressing PTP1B. Cell Metab 2007; 6:307-19.
46. Milne JC, Lambert PD, Schenk S, Carney DP, Smith JJ, Gagne DJ, Jin L, Boss O, Perni RB, Vu CB, Bemis JE, Xie R, Disch JS, Ng PY, Nunes JJ, Lynch AV, Yang H, Galonek H, Israelian K, Choy W, Iffland A, Lavu S, Medvedik O, Sinclair DA, Olefsky JM, Jirousek MR, Elliott PJ, Westphal CH. Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. Nature 2007; 450:712-6.
47. Vaquero A, Scher M, Erdjument-Bromage H, Tempst P, Serrano L, Reinberg D. SIRT1 regulates the histone methyl-transferase SUV39H1 during heterochromatin formation. Nature 2007; 450:440-4.
48. van der Horst A, Tertoolen LG, de Vries-Smits LM, Frye RA, Medema RH, Burgering BM. FOXO4 is acetylated upon peroxide stress and deacetylated by the longevity protein hSir2(SIRT1). J Biol Chem 2004; 279:28873-9.
49. Daitoku H, Hatta M, Matsuzaki H, Aratani S, Ohshima T, Miyagishi M, Nakajima T, Fukamizu A. Silent information regulator 2 potentiates Foxo1-mediated transcription through its deacetylase activity. Proc Natl Acad Sci USA 2004; 101:10042-7.
50. Yuan Z, Zhang X, Sengupta N, Lane WS, Seto E. SIRT1 regulates the function of the Nijmegen breakage syndrome protein. Mol Cell 2007; 27:149-62.
51. Langley E, Pearson M, Faretta M, Bauer UM, Frye RA, Minucci S, Pelicci PG, Kouzarides T. Human SIR2 deacetylates p53 and antagonizes PML/p53-induced cellular senescence. EMBO J 2002; 21:2383-96.
52. Kitamura YI, Kitamura T, Kruse JP, Raum JC, Stein R, Gu W, Accili D. FoxO1 protects against pancreatic beta cell failure through NeuroD and MafA induction. Cell Metab 2005; 2:153-63.
53. Potente M, Urbich C, Sasaki K, Hofmann WK, Heeschen C, Aicher A, Kollipara R, DePinho RA, Zeiher AM, Dimmeler S. Involvement of Foxo transcription factors in angiogenesis and postnatal neovascularization. J Clin Invest 2005; 115:2382-92.
54. Paik JH, Kollipara R, Chu G, Ji H, Xiao Y, Ding Z, Miao L, Tothova Z, Horner JW, Carrasco DR, Jiang S, Gilliland DG, Chin L, Wong WH, Castrillon DH, DePinho RA. FoxOs are lineage-restricted redundant tumor suppressors and regulate endothelial cell homeostasis. Cell 2007; 128:309-23.
55. Yang Y, Hou H, Haller EM, Nicosia SV, Bai W. Suppression of FOXO1 activity by FHL2 through SIRT1-mediated deacetylation. EMBO J 2005; 24:1021-32.
56. Frescas D, Valenti L, Accili D. Nuclear trapping of the forkhead transcription factor FoxO1 via Sirt-dependent deacetylation promotes expression of glucogenetic genes. J Biol Chem 2005; 280:20589-95.
57. Matsuzaki H, Daitoku H, Hatta M, Aoyama H, Yoshimochi K, Fukamizu A. Acetylation of Foxo1 alters its DNA-binding ability and sensitivity to phosphorylation. Proc Natl Acad Sci USA 2005; 102:11278-83.
58. Lonard DM, O'Malley BW. Nuclear receptor coregulators: judges, juries, and executioners of cellular regulation. Mol Cell 2007; 27:691-700.
59. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. N Engl J Med 1993; 329:2002-12.
60. Dimmeler S, Haendeler J, Nehls M, Zeiher AM. Suppression of apoptosis by nitric oxide via inhibition of interleukin-1beta-converting enzyme (ICE)-like and cysteine protease protein (CPP)-32-like proteases. J Exp Med 1997; 185:601-7.
61. Ziche M, Morbidelli L, Masini E, Amerini S, Granger HJ, Maggi CA, Geppetti P, Ledda F. Nitric oxide mediates angiogenesis in vivo and endothelial cell growth and migration in vitro promoted by substance P. J Clin Invest 1994; 94:2036-44.
62. Murohara T, Asahara T, Silver M, Bauters C, Masuda H, Kalka C, Kearney M, Chen D, Symes JF, Fishman MC, Huang PL, Isner JM. Nitric oxide synthase modulates angiogenesis in response to tissue ischemia. J Clin Invest 1998; 101:2567-78.
63. Papapetropoulos A, Garcia-Cardena G, Madri JA, Sessa WC. Nitric oxide production contributes to the angiogenic properties of vascular endothelial growth factor in human endothelial cells. J Clin Invest 1997; 100:3131-9.
64. Aicher A, Heeschen C, Mildner-Rihm C, Urbich C, Ihling C, Technau-Ihling K, Zeiher AM, Dimmeler S. Essential role of endothelial nitric oxide synthase for mobilization of stem and progenitor cells. Nat Med 2003; 9:1370-6.
65. Takata T, Ishikawa F. Human Sir2-related protein SIRT1 associates with the bHLH repressors HES1 and HEY2 and is involved in HES1- and HEY2-mediated transcriptional repression. Biochem Biophys Res Commun 2003; 301:250-7.
66. Fischer A, Schumacher N, Maier M, Sendtner M, Gessler M. The Notch target genes Hey1 and Hey2 are required for embryonic vascular development. Genes Dev 2004; 18:901-11.
67. Hellstrom M, Phng LK, Hofmann JJ, Wallgard E, Coultas L, Lindblom P, Alva J, Nilsson AK, Karlsson L, Gaiano N, Yoon K, Rossant J, Iruela-Arispe ML, Kalen M, Gerhardt H, Betsholtz C. Dll4 signalling through Notch1 regulates formation of tip cells during angiogenesis. Nature 2007; 445:776-80.
68. Siekmann AF, Lawson ND. Notch signalling limits angiogenic cell behaviour in developing zebrafish arteries. Nature 2007; 445:781-4.
69. Suchting S, Freitas C, le Noble F, Benedito R, Breant C, Duarte A, Eichmann A. The Notch ligand Delta-like 4 negatively regulates endothelial tip cell formation and vessel branching. Proc Natl Acad Sci USA 2007; 104:3225-30.
70. Noguera-Troise I, Daly C, Papadopoulos NJ, Coetzee S, Boland P, Gale NW, Lin HC, Yancopoulos GD, Thurston G. Blockade of Dll4 inhibits tumour growth by promoting non-productive angiogenesis. Nature 2006; 444:1032-7.
71. Ridgway J, Zhang G, Wu Y, Stawicki S, Liang WC, Chanthery Y, Kowalski J, Watts RJ, Callahan C, Kasman I, Singh M, Chien M, Tan C, Hongo JA, de Sauvage F, Plowman G, Yan M. Inhibition of Dll4 signalling inhibits tumour growth by deregulating angiogenesis. Nature 2006; 444:1083-7.
72. Hiraoka N, Allen E, Apel IJ, Gyetko MR, Weiss SJ. Matrix metalloproteinases regulate neovascularization by acting as pericellular fibrinolysins. Cell 1998; 95:365-77.
73. Yana I, Sagara H, Takaki S, Takatsu K, Nakamura K, Nakao K, Katsuki M, Taniguchi S, Aoki T, Sato H, Weiss SJ, Seiki M. Crosstalk between neovessels and mural cells directs the site-specific expression of MT1-MMP to endothelial tip cells. J Cell Sci 2007; 120:1607-14.
74. Kume S, Haneda M, Kanasaki K, Sugimoto T, Araki S, Isshiki K, Isono M, Uzu T, Guarente L, Kashiwagi A, Koya D. SIRT1 inhibits transforming growth factor beta-induced apoptosis in glomerular mesangial cells via Smad7 deacetylation. J Biol Chem 2007; 282:151-8.
75. Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, Pistell PJ, Poosala S, Becker KG, Boss O, Gwinn D, Wang M, Ramaswamy S, Fishbein KW, Spencer RG, Lakatta EG, Le Couteur D, Shaw RJ, Navas P, Puigserver P, Ingram DK, de Cabo R, Sinclair DA. Resveratrol improves health and survival of mice on a high-calorie diet. Nature 2006; 444:337-42.
76. Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P, Geny B, Laakso M, Puigserver P, Auwerx J. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell 2006; 127:1109-22.
77. Denu JM. The Sir 2 family of protein deacetylases. Curr Opin Chem Biol 2005; 9:431-40.
78. J G, Cq W, Hh F, Hy D, Xl X, Ym X, By W, Dj H. Effects of resveratrol on endothelial progenitor cells and their contributions to reendothelialization in intima-injured rats. J Cardiovasc Pharmacol 2006; 47:711-21.
79. Wang XB, Huang J, Zou JG, Su EB, Shan QJ, Yang ZJ, Cao KJ. Effects of resveratrol on number and activity of endothelial progenitor cells from human peripheral blood. Clin Exp Pharmacol Physiol 2007; 34:1109-15.
80. Balestrieri ML, Schiano C, Felice F, Casamassimi A, Balestrieri A, Milone L, Servillo L, Napoli C. Effect of low doses of red wine and pure resveratrol on circulating endothelial progenitor cells. J Biochem 2008; 143:179-86.
81. Jain RK, Duda DG, Clark JW, Loeffler JS. Lessons from phase III clinical trials on anti-VEGF therapy for cancer. Nat Clin Pract Oncol 2006; 3:24-40.
82. Saunders LR, Verdin E. Sirtuins: critical regulators at the crossroads between cancer and aging. Oncogene 2007; 26:5489-504.
83. Fischer C, Jonckx B, Mazzone M, Zacchigna S, Loges S, Pattarini L, Chorianopoulos E, Liesenborghs L, Koch M, De Mol M, Autiero M, Wyns S, Plaisance S, Moons L, van Rooijen N, Giacca M, Stassen JM, Dewerchin M, Collen D, Carmeliet P. Anti-PlGF inhibits growth of VEGF(R)-inhibitor-resistant tumors without affecting healthy vessels. Cell 2007; 131:463-75.