HHS Public Access

Author manuscript

Atherosclerosis. Author manuscript; available in PMC 2021 June 12.

Published in final edited form as:

Atherosclerosis. 2021 May ; 324: 117–120. doi:10.1016/j.atherosclerosis.2021.03.034.

Targeting the phenotypic switch of vascular smooth muscle cells to tackle atherosclerosis

Urna Kansakar[a], **Stanislovas S. Jankauskas**[a,b], **Jessica Gambardella**[a,b,c], **Gaetano Santulli**[a,b,c,d,*]

[a]Department of Molecular Pharmacology, Albert Einstein College of Medicine, New York City, 10461, NY, United States

[b]Department of Medicine (Division of Cardiology), Albert Einstein College of Medicine – Montefiore University Hospital, New York City, 10461, NY, United States

[c]Department of Advanced Biomedical Sciences, "Federico II" University, Naples, 80131, Italy

[d]International Translational Research and Medical Education (ITME), Naples, 80100, Italy

Keywords

VSMC; Phenotype switch; Atherosclerosis; MYOCD; KFL4; OCT4; TDG; p27

Vascular smooth muscle cells (VSMCs) play fundamental roles in the pathophysiology of atherosclerosis. A crucial step in VSMC biology is the switch from contractile (differentiated, quiescent, non-migratory) to synthetic (dedifferentiated, proliferative, migratory) phenotypes (Fig. 1). The exact mechanisms underlying VSMC phenotypic switch are not fully known and various pieces of information have been recently added to this puzzle [1–5]. For instance, in this issue of *Atherosclerosis*, Bente Halvorsen's team elegantly demonstrates that Neil3, known to participate in DNA repair, can mediate VSMC phenotypic switch via non-canonical mechanisms [6].

Intriguingly, Neil3 deficiency was shown for the first time to trigger a shift in VSMC phenotype towards a proliferating, lipid-accumulating, and secretory macrophage-like cell phenotype, without major changes in DNA damage [6].

We report herein a concise overview of the main mechanisms regulating VSMC phenotypic switch, mainly relying on transcription factors and epigenetic mechanisms.

1. Regulation of transcriptional activity in VSMC phenotypic switch

The main transcription factors involved in VSMC phenotypic switch are myocardin (MYOCD), Krüppel-like factor 4 (KLF4), and octamer binding transcription factor (Oct4).

*Corresponding author. Albert Einstein College of Medicine, New York City, 10461, NY, United States. gsantulli001@gmail.com (G. Santulli).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

MYOCD, a potent cardiac and smooth muscle tissue-specific transcriptional coactivator of serum response factor (SRF), plays a vital role in inducing smooth muscle differentiation. Numerous downstream muscle-specific genes including *Sm22α*, *Calponin*, and *Acta-2* are activated following the binding of MYOCD to the SRF on the CArG DNA motifs [7]. MYOCD-related transcriptional factors A and B (MRTFs) activate SRF-driven transcription. Several studies have shown that the overexpression of MYOCD and MRTFs induces anti-proliferative effects [8] while their inactivation promotes cell growth and oncogenesis [9]. Equally important, reduction of MRTFs inhibits normal cell cycle progression resulting in an anti-proliferative effect [10]. As a result of this effect, changes such as shortening of G_1 phase and extension of S and G_2 phase occur under normal growth conditions, whereas under serum-starved conditions there is aberrant entry into the S and G_2 phases without any subsequent cell division [11]. MYOCD, along with DNA repair factors like thymine-DNA glycosylase (TDG), recognizes DNA damage in actively expressed areas of the genome and repairs the region-specific DNA [12,13]. Proliferating cells have high expression of TDG and thus instructs the cells to repair DNA mismatches. Moreover, proliferating smooth muscle cells express increased levels of TDG that inhibits both the antiproliferative and promyogenic activities of MYOCD [14].

KLF4 plays an essential role in VMSC proliferation and differentiation [15]. Among the numerous transcriptional regulators that mediate responses to inflammation, proliferation, and differentiation in vascular tissues, KLF family of zinc finger transcription factors have been gaining attention, especially KLF4. There are three different domains of KLF4: transcriptional repression, transcriptional activation, and DNA-binding domains. There are speculations that KLF4 might have anti-proliferative properties because increased levels of KLF4 in numerous cell types have shown growth arrest, reduced cell cycle progression, and inhibition of DNA synthesis [16]. KLF4 regulates gene expression by recognizing and binding to GC-rich DNA sequences or CACCC (GT-box) through its DNA-binding domain. However, studies have demonstrated that KLF4 may transition from pro-cell survival to pro-cell death in certain circumstances [17]. After gamma-irradiation-induced DNA damage [18], KLF4 expression has been shown to be activated in a p53 dependent fashion. The role of p53 is to induce an arrest in the G [1] and S phase of the cell cycle following DNA damage, thereby preventing the irradiated cell to undergo apoptosis [18].

Oct4 is a transcription factor primarily expressed in germline cells that is required to maintain embryonic stem cell (ESC) pluripotency. Oct4 transcription plays a critical role for inner cell mass of blastocysts formation, and loss of expression of Oct4 is associated with spontaneous ESC differentiation; moreover, Oct4 regulates VSMC phenotypic transition and plays an important role in the pathogenesis of atherosclerosis [5,19]. Downregulation of Oct4 contributes to cell cycle regulation of ESCs by blocking G_0/G_1 cycle and preventing proliferation. Further, Oct4 downregulation induces p21 in ESC; inhibition of CDK2 induces G_1 phase arrest followed by apoptosis or differentiation of impaired ESC and causes DNA damage response [20].

2. Role of epigenetics and non-coding RNAs in VSMC phenotypic switch

Mounting evidence has demonstrated that VSMC phenotype switching is regulated by non-coding RNAs, including miRNAs [21], small (approximately 22 nucleotides) single-stranded non-coding RNA molecules that repress protein expression via binding to the 3' untranslated region of specific mRNAs and either blocking the transcription or promoting mRNA degradation [22].

The contractile phenotype of VSMCs is sustained by miR-143/145 and miR-1; miR-1 expression is triggered by the master regulator of contractile phenotype, MYOCD [23,24]; miR-1 targets the 3′UTR of Kruppel-like factor 4 (*Klf 4*) – a transcriptional factor that inhibits MYOCD and induces the expression of genes switching VSMCs to a macrophage-like phenotype [24,25]. Moreover, miR-1 limits VSMC proliferation targeting Proto-oncogene serine/threonine-protein kinase Pim-1 and Histone deacetylase 4 (HDAC4) [23]. Some data suggest that an augmented miR-1 expression may also play a role as a negative feedback loop [26].

Similarly, miR-143/145 expression mirrors VSMC differentiation [27]. Their expression is driven by serum response factor (SRF) – an indispensable coactivator of MYOCD [28,29]. *In vivo* experiments demonstrated that miR-143/145 deficiency results in VSMCs being locked in the synthetic phenotype [27]. miR-143/145 can target and downregulate *Klf4*, ETS Like-1 protein (*Elk-1*), and angiotensin converting enzyme (*Ace*) [27,28]. Moreover, miR-143/145 downregulation by cholesterol was demonstrated *in vitro*, and VSMCs with a low miR-143/145 expression were found in atherosclerotic plaques *in* vivo [28,30].

miR-221 promotes cell proliferation via repression of $p27^{Kip1}$ and inhibits the contractile phenotype via targeting Proto-oncogene c-kit and [31]. VSMCs proliferation is also induced by miR-222 which targets the 3′UTR of $p27^{Kip1}$ and $p57^{Kip2}$ [32]. When both miR-221 and miR-222 are upregulated, they can trigger a transformation of VSMCs in an osteogenic phenotype, characterized by high calcification activity [33]; miR-21 and miR-124 were also found to cause dedifferentiation of VSMCs targeting Specificity protein 1 (*Sp1*) expression [34].

Several miRNAs were shown to play an opposite role, favoring the contractile phenotype. One of the most potent factors in this sense is Platelet derived growth factor BB (PDGF-BB) [35–38]: PDGF-BB induces expression of miR-24 and miR-26a and the inhibition of these miRNAs mitigated PDGF-BB induced proliferation and dedifferentiation of VSMCs [31,39,40]. For instance, miR-24 was shown to suppress the contractile phenotype of VSMCs via repression of Tribbles-like protein-3 (Trb3), a negative regulator of NFκB, which in turn downregulates MYOCD transcription [39,41].

Another class of non-coding RNAs – long-non-coding RNA (lncRNAs) – was recently identified as a regulator of VSMCs phenotype. Among them are SMILR and MALAT1 [42]; the mechanism of their action is not fully understood, however, most likely it involves "sponging" or acting as a decoy for miRNAs [43]. Other lncRNAs that have been shown to modulate VSMC biology are CARMN, PEBP1P2, PVT1, NEAT1, AK098656, and ANRIL [2,44–48].

Epigenetic mechanisms also play significant roles in establishing the VSMCs phenotype. The expression of key genes defining the contractile phenotype of VSMCs – *Acta2* and *Myh11* – are controlled via histone acetylation in SRF binding regions [49]. MYOCD and KLF4 activities heavily rely on HDACs recruitment [12,13]. Methylation of histones H3 and H4 also increases SRF binding to its target regions [12]. Interestingly, di-methylation of lysine 4 of histone H3 (H3K4me2) in *Myh11* could be used as a highly specific marker of non-differentiated VSMCs both in human and mice [50].

Financial support

The Santulli Lab is supported in part by the National Institutes of Health (NIH: R01-DK123259, R01-HL146691, R01-HL159062, R01-DK033823, R56-AG066431, T32-HL144456, R00-DK107895, to G.S.), by the Irma T. Hirschl and Monique Weill-Caulier Trusts (to G.S.), and by the American Heart Association (AHA: 21POST836407 to J.S.S and 20POST35211151 to J.G).

References

[1]. Hartman RJG, Owsiany K, Ma L, Koplev S, Hao K, Slenders L, et al., Sex-stratified gene regulatory networks reveal female key driver genes of atherosclerosis involved in smooth muscle cell phenotype switching, Circulation 143 (7) (2021) 713–726. [PubMed: 33499648]

[2]. Vacante F, Rodor J, Lalwani MK, Mahmoud AD, Bennett M, De Pace AL, et al., CARMN loss regulates smooth muscle cells and accelerates atherosclerosis in mice, Circ. Res (2021).

[3]. Pan H, Xue C, Auerbach BJ, Fan J, Bashore AC, Cui J, et al., Single-cell genomics reveals a novel cell state during smooth muscle cell phenotypic switching and potential therapeutic targets for atherosclerosis in mouse and human, Circulation 142 (21) (2020) 2060–2075. [PubMed: 32962412]

[4]. Riascos-Bernal DF, Chinnasamy P, Gross JN, Almonte V, Egana-Gorrono L, Parikh D, et al., Inhibition of smooth muscle beta-catenin hinders neointima formation after vascular injury, Arterioscler. Thromb. Vasc. Biol 37 (5) (2017) 879–888. [PubMed: 28302627]

[5]. Alencar GF, Owsiany KM, Karnewar S, Sukhavasi K, Mocci G, Nguyen AT, et al., Stem cell pluripotency genes Klf4 and Oct4 regulate complex SMC phenotypic changes critical in late-stage atherosclerotic lesion pathogenesis, Circulation 142 (21) (2020) 2045–2059. [PubMed: 32674599]

[6]. Quiles-Jiménez A, Gregersen I, Segers FM, Skarpengland T, Kroustallaki P, Yang K, et al., DNA glycosylase Neil3 regulates vascular smooth muscle cell biology during atherosclerosis development, Atherosclerosis (2021). In press.

[7]. Xia XD, Zhou Z, Yu XH, Zheng XL, Tang CK, Myocardin: a novel player in atherosclerosis, Atherosclerosis 257 (2017) 266–278. [PubMed: 28012646]

[8]. Chen J, Kitchen CM, Streb JW, Miano JM, Myocardin: a component of a molecular switch for smooth muscle differentiation, J. Mol. Cell. Cardiol 34 (10) (2002) 1345–1356. [PubMed: 12392995]

[9]. Milyavsky M, Shats I, Cholostoy A, Brosh R, Buganim Y, Weisz L, et al., Inactivation of myocardin and p16 during malignant transformation contributes to a differentiation defect, Canc. Cell 11 (2) (2007) 133–146.

[10]. Shaposhnikov D, Kuffer C, Storchova Z, Posern G, Myocardin related transcription factors are required for coordinated cell cycle progression, Cell Cycle 12 (11) (2013) 1762–1772. [PubMed: 23656782]

[11]. Tang RH, Zheng XL, Callis TE, Stansfield WE, He J, Baldwin AS, et al., Myocardin inhibits cellular proliferation by inhibiting NF-kappaB(p65)-dependent cell cycle progression, Proc. Natl. Acad. Sci. U. S. A 105 (9) (2008) 3362–3367. [PubMed: 18296632]

[12]. McDonald OG, Wamhoff BR, Hoofnagle MH, Owens GK, Control of SRF binding to CArG box chromatin regulates smooth muscle gene expression in vivo, J. Clin. Invest 116 (1) (2006) 36–48. [PubMed: 16395403]

[13]. Cao D, Wang Z, Zhang CL, Oh J, Xing W, Li S, et al., Modulation of smooth muscle gene expression by association of histone acetyltransferases and deacetylases with myocardin, Mol. Cell Biol 25 (1) (2005) 364–376. [PubMed: 15601857]

[14]. Zhou J, Blue EK, Hu G, Herring BP, Thymine DNA glycosylase represses myocardin-induced smooth muscle cell differentiation by competing with serum response factor for myocardin binding, J. Biol. Chem 283 (51) (2008) 35383–35392. [PubMed: 18945672]

[15]. Yang C, Xiao X, Huang L, Zhou F, Chen LH, Zhao YY, et al., Role of Kruppel-like factor 4 in atherosclerosis, Clin. Chim. Acta 512 (2021) 135–141. [PubMed: 33181148]

[16]. Zheng B, Han M, Wen JK, Role of Kruppel-like factor 4 in phenotypic switching and proliferation of vascular smooth muscle cells, IUBMB Life 62 (2) (2010) 132–139. [PubMed: 20073036]

[17]. Li Z, Zhao J, Li Q, Yang W, Song Q, Li W, et al., KLF4 promotes hydrogen-peroxide-induced apoptosis of chronic myeloid leukemia cells involving the bcl-2/bax pathway, Cell Stress Chaperones 15 (6) (2010) 905–912. [PubMed: 20401760]

[18]. Yoon HS, Chen X, Yang VW, Kruppel-like factor 4 mediates p53-dependent G1/S cell cycle arrest in response to DNA damage, J. Biol. Chem 278 (4) (2003) 2101–2105. [PubMed: 12427745]

[19]. Cherepanova OA, Gomez D, Shankman LS, Swiatlowska P, Williams J, Sarmento OF, et al., Activation of the pluripotency factor OCT4 in smooth muscle cells is atheroprotective, Nat. Med 22 (6) (2016) 657–665. [PubMed: 27183216]

[20]. Neganova I, Vilella F, Atkinson SP, Lloret M, Passos JF, von Zglinicki T, et al., An important role for CDK2 in G1 to S checkpoint activation and DNA damage response in human embryonic stem cells, Stem Cell 29 (4) (2011) 651–659.

[21]. Santulli G, microRNAs distinctively regulate vascular smooth muscle and endothelial cells: functional implications in angiogenesis, atherosclerosis, and in-stent restenosis, Adv. Exp. Med. Biol 887 (2015) 53–77. [PubMed: 26662986]

[22]. Bartel DP, Metazoan MicroRNAs, Cell 173 (1) (2018) 20–51. [PubMed: 29570994]

[23]. Chen J, Yin H, Jiang Y, Radhakrishnan SK, Huang ZP, Li J, et al., Induction of microRNA-1 by myocardin in smooth muscle cells inhibits cell proliferation, Arterioscler. Thromb. Vasc. Biol 31 (2) (2011) 368–375. [PubMed: 21051663]

[24]. Xie C, Huang H, Sun X, Guo Y, Hamblin M, Ritchie RP, et al., MicroRNA-1 regulates smooth muscle cell differentiation by repressing Kruppel-like factor 4, Stem Cell. Dev 20 (2) (2011) 205–210.

[25]. Shankman LS, Gomez D, Cherepanova OA, Salmon M, Alencar GF, Haskins RM, et al., KLF4-dependent phenotypic modulation of smooth muscle cells has a key role in atherosclerotic plaque pathogenesis, Nat. Med 21 (6) (2015) 628–637. [PubMed: 25985364]

[26]. Jiang Y, Yin H, Zheng XL, MicroRNA-1 inhibits myocardin-induced contractility of human vascular smooth muscle cells, J. Cell. Physiol 225 (2) (2010) 506–511. [PubMed: 20458751]

[27]. Boettger T, Beetz N, Kostin S, Schneider J, Kruger M, Hein L, et al., Acquisition of the contractile phenotype by murine arterial smooth muscle cells depends on the Mir143/145 gene cluster, J. Clin. Invest 119 (9) (2009) 2634–2647. [PubMed: 19690389]

[28]. Cordes KR, Sheehy NT, White MP, Berry EC, Morton SU, Muth AN, et al., miR-145 and miR-143 regulate smooth muscle cell fate and plasticity, Nature 460 (7256) (2009) 705–710. [PubMed: 19578358]

[29]. Li S, Wang DZ, Wang Z, Richardson JA, Olson EN, The serum response factor coactivator myocardin is required for vascular smooth muscle development, Proc. Natl. Acad. Sci. U. S. A 100 (16) (2003) 9366–9370. [PubMed: 12867591]

[30]. Vengrenyuk Y, Nishi H, Long X, Ouimet M, Savji N, Martinez FO, et al., Cholesterol loading reprograms the microRNA-143/145-myocardin axis to convert aortic smooth muscle cells to a dysfunctional macrophage-like phenotype, Arterioscler. Thromb. Vasc. Biol 35 (3) (2015) 535–546. [PubMed: 25573853]

[31]. Davis BN, Hilyard AC, Nguyen PH, Lagna G, Hata A, Induction of microRNA-221 by platelet-derived growth factor signaling is critical for modulation of vascular smooth muscle phenotype, J. Biol. Chem 284 (6) (2009) 3728–3738. [PubMed: 19088079]

[32]. Liu X, Cheng Y, Zhang S, Lin Y, Yang J, Zhang C, A necessary role of miR-221 and miR-222 in vascular smooth muscle cell proliferation and neointimal hyperplasia, Circ. Res 104 (4) (2009) 476–487. [PubMed: 19150885]

[33]. Mackenzie NC, Staines KA, Zhu D, Genever P, Macrae VE, miRNA-221 and miRNA-222 synergistically function to promote vascular calcification, Cell Biochem. Funct 32 (2) (2014) 209–216. [PubMed: 24604335]

[34]. Yang G, Pei Y, Cao Q, Wang R, MicroRNA-21 represses human cystathionine gamma-lyase expression by targeting at specificity protein-1 in smooth muscle cells, J. Cell. Physiol 227 (9) (2012) 3192–3200. [PubMed: 22034194]

[35]. Lu QB, Wan MY, Wang PY, Zhang CX, Xu DY, Liao X, et al., Chicoric acid prevents PDGF-BB-induced VSMC dedifferentiation, proliferation and migration by suppressing ROS/NFkappaB/mTOR/P70S6K signaling cascade, Redox Biol 14 (2018) 656–668. [PubMed: 29175753]

[36]. Song W, Gao K, Huang P, Tang Z, Nie F, Jia S, et al., Bazedoxifene inhibits PDGF-BB induced VSMC phenotypic switch via regulating the autophagy level, Life Sci. 259 (2020) 118397. [PubMed: 32896557]

[37]. Inui H, Kitami Y, Tani M, Kondo T, Inagami T, Differences in signal transduction between platelet-derived growth factor (PDGF) alpha and beta receptors in vascular smooth muscle cells. PDGF-BB is a potent mitogen, but PDGF-AA promotes only protein synthesis without activation of DNA synthesis, J. Biol. Chem 269 (48) (1994) 30546–30552. [PubMed: 7982973]

[38]. Azahri NS, Di Bartolo BA, Khachigian LM, Kavurma MM, Sp1, acetylated histone-3 and p300 regulate TRAIL transcription: mechanisms of PDGF-BB-mediated VSMC proliferation and migration, J. Cell. Biochem 113 (8) (2012) 2597–2606. [PubMed: 22415975]

[39]. Chan MC, Hilyard AC, Wu C, Davis BN, Hill NS, Lal A, et al., Molecular basis for antagonism between PDGF and the TGFbeta family of signalling pathways by control of miR-24 expression, EMBO J. 29 (3) (2010) 559–573. [PubMed: 20019669]

[40]. Yang X, Dong M, Wen H, Liu X, Zhang M, Ma L, et al., MiR-26a contributes to the PDGF-BB-induced phenotypic switch of vascular smooth muscle cells by suppressing Smad1, Oncotarget 8 (44) (2017) 75844–75853. [PubMed: 29100273]

[41]. Yoshida T, Yamashita M, Horimai C, Hayashi M, Smooth muscle-selective inhibition of nuclear factor-kappaB attenuates smooth muscle phenotypic switching and neointima formation following vascular injury, J Am Heart Assoc 2 (3) (2013), e000230. [PubMed: 23702880]

[42]. Mahmoud AD, Ballantyne MD, Miscianinov V, Pinel K, Hung J, Scanlon JP, et al., The human-specific and smooth muscle cell-enriched LncRNA SMILR promotes proliferation by regulating mitotic CENPF mRNA and drives cell-cycle progression which can Be targeted to limit vascular remodeling, Circ. Res 125 (5) (2019) 535–551. [PubMed: 31339449]

[43]. Paraskevopoulou MD, Hatzigeorgiou AG, Analyzing MiRNA-LncRNA interactions, Methods Mol. Biol 1402 (2016) 271–286. [PubMed: 26721498]

[44]. Hu DJ, Li ZY, Zhu YT, Li CC, Overexpression of long noncoding RNA ANRIL inhibits phenotypic switching of vascular smooth muscle cells to prevent atherosclerotic plaque development in vivo, Aging (Albany NY) 13 (3) (2020) 4299–4316. [PubMed: 33411680]

[45]. He X, Lian Z, Yang Y, Wang Z, Fu X, Liu Y, et al., Long non-coding RNA PEBP1P2 suppresses proliferative VSMCs phenotypic switching and proliferation in atherosclerosis, Mol. Ther. Nucleic Acids 22 (2020) 84–98. [PubMed: 32916601]

[46]. Zhang Z, Zou G, Chen X, Lu W, Liu J, Zhai S, et al., Knockdown of lncRNA PVT1 inhibits vascular smooth muscle cell apoptosis and extracellular matrix disruption in a murine abdominal aortic aneurysm model, Mol. Cell 42 (3) (2019) 218–227.

[47]. Ahmed ASI, Dong K, Liu J, Wen T, Yu L, Xu F, et al., Long noncoding RNA NEAT1 (nuclear paraspeckle assembly transcript 1) is critical for phenotypic switching of vascular smooth muscle cells, Proc. Natl. Acad. Sci. U. S. A 115 (37) (2018) E8660–E8667. [PubMed: 30139920]

[48]. Jin L, Lin X, Yang L, Fan X, Wang W, Li S, et al., AK098656, a novel vascular smooth muscle cell-dominant long noncoding RNA, promotes hypertension, Hypertension 71 (2) (2018) 262–272. [PubMed: 29279317]

[49]. Manabe I, Owens GK, Recruitment of serum response factor and hyperacetylation of histones at smooth muscle-specific regulatory regions during differentiation of a novel P19-derived in vitro

smooth muscle differentiation system, Circ. Res 88 (11) (2001) 1127–1134. [PubMed: 11397778]

[50]. Gomez D, Shankman LS, Nguyen AT, Owens GK, Detection of histone modifications at specific gene loci in single cells in histological sections, Nat. Methods 10 (2) (2013) 171–177. [PubMed: 23314172]

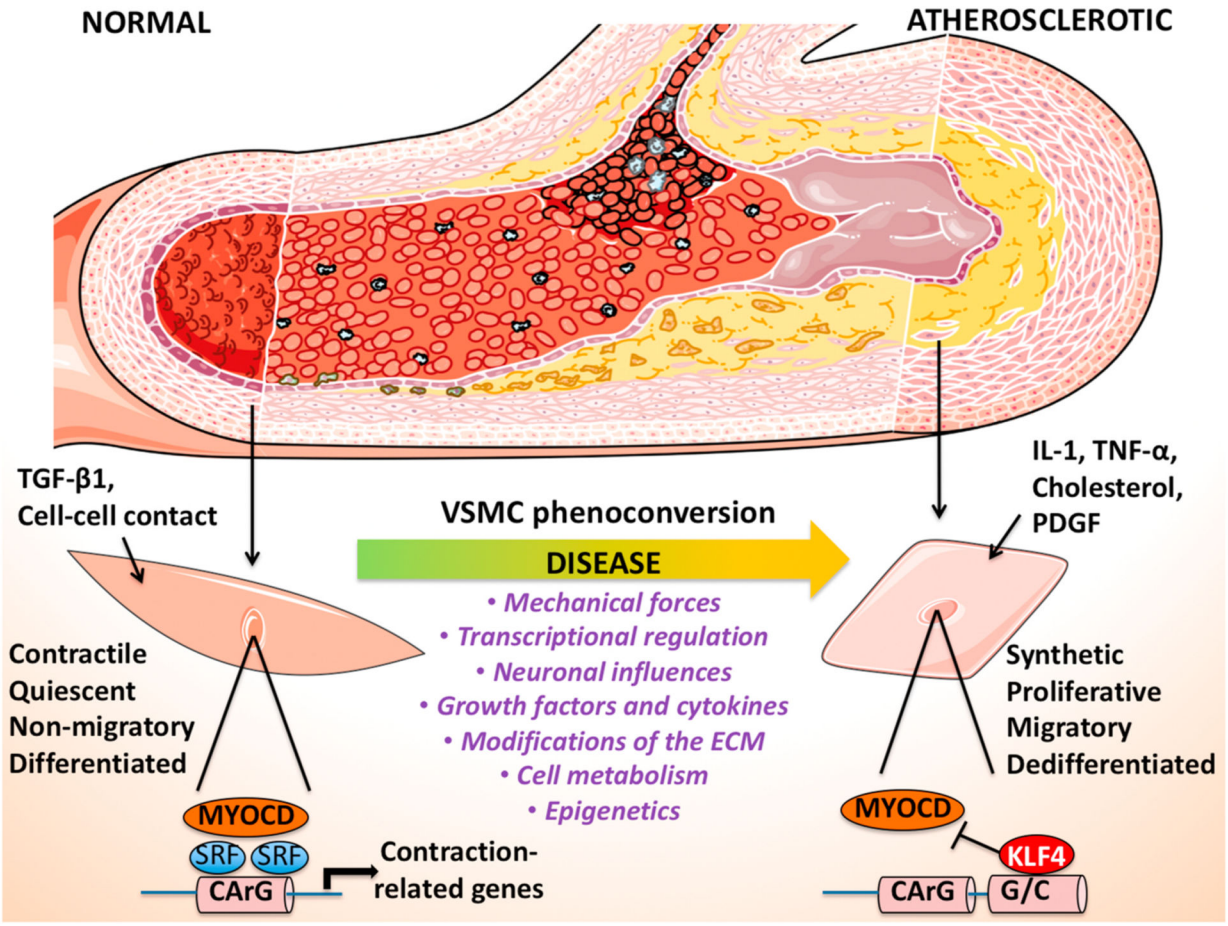

Fig. 1.

VSMC phenotypic switch.

The cartoon summarizes the main mechanisms underlying the phenotypic switch of vascular smooth muscle cells (VSMCs) from contractile to synthetic. ECM: extracellular matrix; IL-1: interleukin-1; KLF4: Krüppel-like factor 4; MYOCD: myocardin; PDGF: platelet-derived growth factor; TGF-β1: transforming growth factor beta 1; TNF-α: Tumor necrosis factor alpha; SRF: serum response factor.