Mechanical Stretch Simulates Proliferation of Venous Smooth Muscle Cells Through Activation of the Insulin-Like Growth Factor-1 Receptor

Jizhong Cheng, Jie Du

Objective—Activation and proliferation of vascular smooth muscle cells (VSMCs) occur in the venous neointima of vein grafts. VSMCs in a grafted vein are subjected to mechanical stretch; our goal is to understand the essential mechanical stretch-regulated signals that influence VSMCs during neointimal formation in vein grafts.

Methods and Results—In cultured vein VSMCs, mechanical stretch induces proliferation and upregulation of both IGF-1 and IGF-1R. Stretch of VSMCs sustained tyrosine phosphorylation of both IGF-1R and its substrate, IRS-1; these responses were related to mechanical stretch-induced activation of Src and autocrine IGF-1 production. Mechanical stretch-activated IGF-1R is functional because there is a prolonged activation of IRS-1-associated phosphatidylinositol-3 kinase (PI3K). When we knocked out IGF-1R, the mechanical stretch-induced increase in VSMC proliferation was blocked. To link mechanical stretch-activated IGF-1R cell signaling to venous VSMC proliferation in vivo, we also studied a vein graft model. Tamoxifen-inducible null deletion of IGF-1R in mice reduced the formation of neointima in the vein graft.

Conclusions—Our results demonstrate for the first time that mechanical stretch activates IGF-1/IGF-1R signals in venous VSMCs, and we have uncovered a signaling pathway that leads to neointima formation in vivo. (Arterioscler Thromb Vasc Biol. 2007;27:000-000.)

Key Words: ●●●

Vein grafts in a coronary bypass graft or in the venous end of a hemodialysis access often develop obliterative neointima formation. Unfortunately, there is no routinely successful method of preventing this problem. In both cases, there is a sharp increase in physical forces/tension in the vein attributable to higher flow velocities in the arterialized circulation.1,2 There also is turbulent blood flow in grafted veins and at the venous anastomosis.3,4 In fact, these is evidence that mechanical stress stimulates the synthesis or secretion of various bioactive molecules including prostacyclin, nitric oxide synthase, platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and the transcription factors c-Fos/c-Jun.5,6 Failed vein grafts also exhibit increased expression of growth factors such as vascular endothelial growth factor, bFGF, and PDGF. But, it is unclear which of these activated signals is essential or responsible for activation of VSMC proliferation in the grafted vein.

VSMC proliferation can be regulated by multiple growth factors including PDGF, bFGF, thrombin, angiotensin II, and insulin like growth factor-1 (IGF-1).7 IGF-1 has a special role in this response because it regulates progression of VSMCs to the S phase from the G1-phase of the cell cycle; it also functions both as an endocrine and autocrine/paracrine growth factor. For example, downregulation of IGF-1R using antisense IGF-1R cDNA or antisense phosphorothioate oligonucleotides markedly suppresses serum- or growth factor-induced growth responses in cultured VSMCs.8 Neutralizing anti-IGF-1 antibodies will block the growth responses that are observed in response to treatment with angiotensin II, thrombin, bFGF, or serum.8 We have found that growth factors, such as bFGF or PDGF, activate IGF-1R transcription and upregulate IGF-1R density in VSMCs.9,10 Thus, stimulation of an IGF-1/IGF-1R autocrine loop could be pivotal in mediating growth factor–induced activation of VSMCs. In this regard, the level of IGF-1R expression is a critical determinant of the proliferative capacity of cells; a small increment in IGF-1R expression is sufficient to switch cells from a quiescent to a mitogenic state.11 For these reasons, an increase in IGF-1R is likely to be a critical step in VSMC proliferation.

Our goal is to understand the essential signals that influence VSMCs during neointimal formation in vein grafts. We concentrated on examining IGF-1R as a mediator of neointima formation in vein graft and found that mechanical stretch stimulates the expression and activation of both IGF-1 and its...
receptor. The critical role of IGF-1R in vein graft neointima formation was documented in vivo in experiments using a null deletion of IGF-1R. These results support our hypothesis that activation of IGF-1/IGF-1R growth signals by mechanical stretch is responsible for the proliferation of venous VSMCs.

Materials and Methods

Generation of Mice With Tamoxifen-Inducible Null Deletion of IGF-1R
All protocols were approved by the Baylor College of Medicine Institutional Review Board. Transgenic mice with ER-Cre activated by the estrogen analogue, tamoxifen, were from Jackson Laboratory (Bar harbor, Me). Mice with LoxP floxed IGF-1R (exon 3) in a C57BL6 background were bred with these mice.12 Mice with ER-Cre and LoxP-floxed IGF-1R were identified by genotyping (Cre primer, 5′-TGCGTGAGAGCTGGAACCACTGTCG-3′; 5′-CCATGAGTGAACAGAACCTGGTCG-3′; P2, 5′-GCCAGGCTGGAACCTGGTCG-3′; P3, 5′-AGCTGCCAGGCACTCCG-3′; P3, 5′-GCCAGGCTGGAACCTGGTCG-3′; P3, 5′-GCCAGGCTGGAACCTGGTCG-3′). RT-polymerase chain reaction (PCR) products of the 518 bp (ie, P1-P3) corresponded to IGF-1R exon 3 knockout, whereas the 574-bp fragment indicated the intact IGF-1R gene.12 At 3 months, mice with IGF-1R LoxP Cre or control mice (IGF-1R LoxP LoxP) were injected intraperitoneally (i.p.) with 1 or 3 mg tamoxifen (10 mg/mL in corn oil) for 7 days. IGF-1R deletion was detected by RT-PCR, western blot, and immunohistochemistry.

Vein Graft Procedure
Vein grafts were created as described by Zou et al.13 In brief, the right common carotid artery of a male mouse was mobilized and divided. A cuff was placed on both ends of the artery, and the ends were everted over the cuff and ligated with an 8.0 silk ligature. Vena cavae from donor mice were grafted between the 2 ends of the graft. After 4 weeks, the vein grafts were obtained and the intima plus media was measured as the distance between the lumen and the adventitia. Vessel wall thickness was measured as area of the vessel minus that of the lumen using NIH Image Program Image J and AxioVision software. Five cross-sections were examined as described by Hu et al.12

Immunohistochemistry
After removing paraffin and rehydrating, sections were incubated for 30 minutes in 3% H2O2 in methanol at room temperature (RT), washed with double distilled water and PBS, and heated in a microwave to 199°F for 30 minutes. Sections were blocked with 10% goat serum (Vector Laboratories) for 30 minutes and then incubated with primary antibodies. After washing, sections were incubated with a biotinylated goat anti-rabbit secondary antibody (Vector Laboratories) at RT. After washes, sections were incubated with the ABC100 reagent (Vector Laboratories) for 40 minutes. The immunostaining was visualized using a peroxidase substrate kit (Vector Laboratories) according to the manufacturer’s protocol. The sections were also counterstained with hematoxylin, dehydrated in alcohol, cleared with xylene, and examined by light microscopy. For a negative control, the same protocol was used with antigen dilution reagent instead of the primary antibodies. The staining density was analyzed with SigmaScan Pro. 5.0.

Vein Smooth Muscle Cells and Cyclic Stress
Vena cava explants from mice were used to isolate venous VSMCs that were grown in DMEM supplemented with 10% fetal bovine serum at 37°C. Mouse VSMCs stained positively with smooth muscle actin (SMA). To assess mechanical cyclic stretch, 90% confluent vein VSMCs on silicone elastomer-bottomed and collagen-coated plates (Flexcell) were subjected to mechanical stress using the computer-controlled Cyclic Stress Unit (Flexcell 4000) with defined cyclic deformation (60 cycles/min) and 0 to 20% elongation of elastomer-bottomed plates.

Western Blot Analysis
Cells were lysed in RIPA buffer and 30 μg proteins were separated on SDS-polyacrylamide gels and transferred onto polyvinylidene fluoride (PVDF) membranes. Membranes were incubated with specific antibodies as described.14

Measurement of PI3K Activity
PI3K activity in immunoprecipitates was assayed as described.15 Serum-starved vein VSMCs grown on silicone elastomer-bottomed plates were subjected to mechanical stretch at 37°C for various times before cells were lysed and immunoprecipitated with the anti–IRS-1 antibody overnight at 4°C.

BrDU Incorporation
Brdu immunostaining was performed according to the manufacturer’s protocol (Roche).

Statistics
Results are presented as mean±SEM. Comparison between groups was made using 1-way ANOVA; P<0.05 was considered statistically significant.

Results

Mechanical Stretch Stimulates Expression of IGF-1 and Its Receptor
We have shown that IGF-1/IGF-1R is essential for growth factor–induced rat aortic VSMC proliferation.7 To examine whether mechanical stretch stimulates expression of IGF-1 and IGF-1R in vein VSMCs, we measured the time course of IGF-1 and IGF-1R mRNAs; mechanical stretch (10% elongation at 1 Hz) increased the peak mRNA levels of IGF-1R and IGF-1×3.0-fold (2 hours, Figure 1A) and 4.6-fold (8 hours, Figure 1B), respectively. The increase in IGF-1R mRNA was associated with a 2.4-fold increase in IGF-1R protein (Figure 1C). Because VSMCs in a vein graft are subjected to different degrees of mechanical stretch, we also examined the relationship between the degree of mechanical stretch and induction of IGF-1R. Mechanical stretch increases the level of IGF-1R protein in a stretch-dependent fashion, peaking at 10% to 15% elongation (Figure 1D).

Mechanical Stretch Induces Sustained Tyrosine Phosphorylation of IGF-1R
To determine whether mechanical stretch-stimulated expression of IGF-1 and IGF-1R is accompanied by activation of the IGF-1R signaling pathway, we examined tyrosine phosphorylation of IGF-1R. After exposure to cycle stretch, we immunoprecipitated tyrosine phosphorylated proteins using anti–phospho-tyrosine antibody, 4G-10, and then detected PI3K activity in immunoprecipitates as described.15 We have shown that IGF-1/IGF-1R is essential for growth factor–induced rat aortic VSMC proliferation.7 To examine whether mechanical stretch stimulates expression of IGF-1 and IGF-1R in vein VSMCs, we measured the time course of IGF-1 and IGF-1R mRNAs; mechanical stretch (10% elongation at 1 Hz) increased the peak mRNA levels of IGF-1R and IGF-1×3.0-fold (2 hours, Figure 1A) and 4.6-fold (8 hours, Figure 1B), respectively. The increase in IGF-1R mRNA was associated with a 2.4-fold increase in IGF-1R protein (Figure 1C). Because VSMCs in a vein graft are subjected to different degrees of mechanical stretch, we also examined the relationship between the degree of mechanical stretch and induction of IGF-1R. Mechanical stretch increases the level of IGF-1R protein in a stretch-dependent fashion, peaking at 10% to 15% elongation (Figure 1D).

Mechanical Stretch Sustains IGF-1R Activation by IGF-1–Dependent and IGF-1–Independent, c-Src–Mediated Mechanisms
To determine how mechanical stretch activates IGF-1R signaling pathway, we examined its dependency on autocrine
production of IGF-1. VSMCs were exposed to mechanical stretch in the presence or absence of a neutralizing anti–IGF-1 antibody (H9251-IGF-1). Mechanical stretch induced the tyrosine phosphorylation of IGF-1R at early phase (30 minutes); this induction was unaffected by the IGF-1 neutralizing antibody. In contrast, the IGF-1 antibody reduced the stretch-induced late phase (24 hour) tyrosine phosphorylation of IGF-1R and IRS-1 (Figure 2B). These results suggest that autocrine production of IGF-1 mediates the late phase of tyrosine phosphorylation of IGF-1R and IRS-1. In contrast, the early phase of IGF-1R and IRS-1 tyrosine phosphorylation is ligand-independent.

What is the kinase activating the early phase? Mechanical stress can activate tyrosine kinase Src.16 It is also known that c-Src can phosphorylate and activate IGF-1R,17 so we examined whether c-Src mediates the early phase of tyrosine phosphorylation of IGF-1R at early phase (30 minutes); this induction was unaffected by the IGF-1 neutralizing antibody. In contrast, the IGF-1 antibody reduced the stretch-induced late phase (24 hour) tyrosine phosphorylation of IGF-1R and IRS-1 (Figure 2B). These results suggest that autocrine production of IGF-1 mediates the late phase of tyrosine phosphorylation of IGF-1R and IRS-1. In contrast, the early phase of IGF-1R and IRS-1 tyrosine phosphorylation is ligand-independent.

Inhibition of IGF-1R Prevents Mechanical Stretch-Induced VSMC Proliferation

To determine the effect of stretch on vein VSMC proliferation, VSMCs were subjected to stretch. There was a significant increase in BrdU incorporation, which can be inhibited by PI3K inhibitor (Ly294002 or DN p85; Figure 3A) and c-src inhibitor (PP2 or DN src; Figure 3B). To examine how mechanical stretch-activated IGF-1/IGF-1R signaling influences proliferation, we measured IRS-1–associated PI3K activity and phosphorylation of AKT. Even in the absence of exogenous IGF-1, stretch stimulated IRS-1–associated PI3K activity as early as 5 minutes and lasted at least for 60 minutes (Figure 2F). Activated PI3K was accompanied by sustained AKT phosphorylation (supplemental Figure II). Taken together, these results indicate that mechanical stretch sustains the activation of IGF-1R signaling.
Figure 2. Cyclic stretch activates the IGF-1R pathway through IGF-1 production and the tyrosine kinase, c-Src. A, Cyclic stretch induces dual phases of tyrosine phosphorylation of IGF-1R. Venous VSMCs were subjected to cyclic stretch before the cell lysates were prepared and supernatants of the lysates were immunoprecipitated with the anti-tyrosine antibody, 4G10, and blotted with IGF-1R antibodies (*P<0.05 vs control, n=4). B, An IGF-1 neutralizing antibody blocks the cyclic stretch-induced late phase activation of the IGF-1R pathway. VSMCs were pretreated with 2 μg/mL neutralizing IGF-1 antibody (α-IGF-1) for 2 hours and then subjected to cyclic stretch; tyrosine phosphorylated IGF-1R and IRS-1 was detected as in A. C, Cyclic stretch increases c-Src activation. Phosphorylation of tyrosine 416 of c-Src in VSMCs was detected by Western blotting after cyclic stretch. D, c-Src kinase inhibition blunts the cyclic stretch-induced early phase of IGF-1R/IRS-1 activation. VSMCs pretreated with c-Src kinase inhibitor, PP2 (10 μmol/L), for 30 minutes, were subjected to cyclic stretch and cells were collected at different times. IGF-1R and IRS-1 tyrosine phosphorylation were analyzed by immunoprecipitation with anti-phosphotyrosine antibody, 4G10. The results represent values from 4 repeated experiments (*P<0.05 vs control). E, c-Src inhibition suppresses stretch-mediated tyrosine phosphorylation of IGF-1R and IRS-1. VSMCs infected with c-Src were subjected to cyclic stretch for 30 minutes, the tyrosine phosphorylation of IGF-1R and IRS-1 were performed as described in panel D. F, Cyclic stretch increases IRS-1–associated PI3K activity. VSMCs were subjected to cyclic stretch and collected at different times, and the IRS-1–associated PI3K activity was measured as described in Materials and Methods.
stretch increased BrdU-positive cells compared with results in unstretched control cells (Figure 3C). VSMC proliferation attributable to mechanical stretch was suppressed by IGF-1R deletion; BrdU-positive cells decreased to 63% of the value in mechanically stretched control cells (Figure 3C). Thus, stretch induces VSMC proliferation dependent on the IGF-1R. Cre-mediated IGF-1R deletion in VSMCs did not change PDGF receptor and EGF receptor levels (supplemental Figure III).

**IGF-1R Level Is Increased in Neointima in Vein Graft**
In wild-type mice, neointima hyperplasia and thickening of the vessel wall at 4 weeks after placing the vein into aorta had more than 20 to 35 layers of VSMCs; the control vein has only 1 to 2 layers of VSMCs (Figure 4A). In perfusion-fixed tissues, the neointima in a vein graft strongly stained for both IGF-1R (5.9-fold increase versus control vein, n=6, *P*<0.01, Figure 4A) and phospho-AKT (1.9-fold increase, n=6, *P*<0.01, Figure 4B).

**IGF-1R Deletion Reduces Neointima Formation in Vein Grafts**
IGF-1R knockout seriously affects development of mice and causes perinatal mortality. To study how IGF-1R affects neointima formation, we developed an inducible Cre-LoxP system to delete IGF-1R in intact mice (Figure 5A). Genotyping (data not shown) showed offspring mice bearing both the ER-Cre gene and the homozygous LoxP-floxed IGF-1R gene. At 3 months, these mice were injected daily with tamoxifen for 1 week to activate Cre recombinase to delete LoxP-floxed IGF-1R. Tamoxifen treatment significantly reduced IGF-1R in veins (Figure 5B and 5C). Vein grafts were then created in 6 of these mice using vena cavae obtained from other tamoxifen-treated mice. After 4 weeks, the vein grafts were collected for Western blot and immunostaining. There was an 80% decrease in the IGF-1R protein level in the vein graft of IGF-1RFloxP/ER-cre transgenic mice compared with the level in the vein graft of tamoxifen-treated IGF-1RFloxP/ER-cre mice (Figure 5B). Immunostaining of perfusion-fixed tissues showed that activation of Cre eliminated most of the IGF-1R expression in the vein graft (Figure 5D), but PDGF receptor and EGF receptor levels were not affected clearly, consistent with results of the Western blots. There was ∼6.5-fold increase in the number of PCNA (proliferation marker)-positive cells in the neointima of veins in tamoxifen-treated littermate control, IGF-1RFloxP/ER-cre−/− mice compared with values in the vein graft of tamoxifen-treated IGF-1RFloxP/ER-cre−/− transgenic mice.
Accelerated arteriosclerosis can develop rapidly in vein grafts, and this response has been closely linked to increased biomechanical forces related to alterations in blood pressure in the vein (estimated as 0 to 30 mm Hg) compared with arterial pressure (120 mm Hg). The report by Lardenoye et al showed that relief of this pressure with an external stent placed in vein graft model results in much less VSMC accumulation indicating that mechanical stretch of the vein will stimulate proliferation of venous VSMCs. In cultured VSMCs from veins we have demonstrated that mechanical stretch (15% elongation) significantly stimulates VSMC proliferation as well as the expression of an important growth factor pathway namely, the IGF-1/IGF-1R pathway (Figures 4, 2, and 3). Interestingly, Hu et al demonstrated that mechanical stretch can directly alter the conformation of the PDGF receptor and initiates signaling pathways that are normally used by PDGF. They also report that suramin, a drug that binds to many growth factors, can dissociate growth factors from their receptors, and will inhibit neointima formation in a vein graft. These results are consistent with our findings because suramin also inhibits IGF-1 signaling.

What are potential mechanisms for IGF-1/IGF-1R-dependent VSMC proliferation in response to stretch? Mechanical stretch can activate ligand-independent PDGF activation and can stimulate the production of growth factors (PDGF, FGF, and transforming growth factor-β). We and others have shown that there is cross talk between other growth factors and the expression of IGF-1/IGF-1R. Specifically, we found that growth factors increase IGF-1R expression. We and others also find that the absence of IGF-1R results in loss of the PDGF-induced growth response in fibroblast cells. Thus, the finding that mechanical stretch stimulates an IGF-1/IGF-1R pathway suggests that this pathway has a pivotal role in mediating growth factor–induced activation of VSMCs.

How does mechanical stretch activate the IGF-1R? Mechanical stress is known to activate c-Src within seconds, and we found evidence that c-Src is involved in the early phase phosphorylation/activation of IGF-1R in vein VSMCs responding to mechanical stretch. When we inhibited c-Src with PP2, mechanical stretch-induced tyrosine phosphorylation/activation of IGF-1R in vein VSMCs (Figure 5D). The increased phosphorylation of AKT and decreased in p27Kip1 were detected with immunostaining (Figure 5D) and Western blot (supplemental Figure IV) in control vein grafts were absent in vein grafts from IGF-1R null mice. Compared with control mice, neointimal hyperplasia in vein grafts from IGF-1R knockout mice was reduced by at least 44.4% (Figure 5E). The lumen/media ratio was increased by 75% in vein grafts from IGF-1R knockout mice compared with values in vein graft of the control mice (Figure 5F). We conclude that vascular remodeling in a grafted vein depends on IGF-1R.

**Discussion**

Mechanical stretch of veins occurs when a vein is grafted into an artery to create a vascular access or arterial bypass. Stretch is believed to play a major role in activating growth signaling pathways, ultimately leading to proliferation of VSMCs, a key event in the development of neointima in the vein or a vein graft. The mechanisms by which venous VSMCs sense mechanical stretch and activate growth signals leading to VSMC proliferation have not been fully characterized. We investigated IGF-1/IGF-1R signaling because multiple growth factors such as PDGF, bFGF, or angiotensin II exert their growth responses via upregulation of IGF-1 and IGF-1R. The present results demonstrate for the first time that mechanical stretch activates IGF-1R and its downstream signaling pathways on venous VSMCs and in a vein graft in vivo. Our results also identify, for the first time, a cause-effect role for IGF-1R in vascular remodeling in vein grafts.

We have demonstrated that mechanical stretch not only increases the expression of IGF-1R (Figure 1), but it also stimulates and sustains tyrosine phosphorylation of IGF-1R and IRS-1 (Figure 2). Mechanical stretch stimulated tyrosine phosphorylation of IGF-1R and IRS-1 and activation of IRS-1–associated PI3K. In support of this pathway, we found that null deletion of IGF-1R in mice significantly reduces neointimal hyperplasia in vein graft (Figure 5D). Our results, therefore, provide clear evidence that mechanical stretch activates IGF-1R and that this is an essential step stimulating VSMC proliferation.

![Image](image-url)
Figure 5. Knockout of IGF-1R suppresses neointimal formation in the vein of a vein graft. A, Schematic representation of the construct used to create a tamoxifen-induced knockout of IGF-1R. B and C, Tamoxifen induces knockout of IGF-1R in the vein of a vein graft. IGF-1R<sup>loxP<sup>+/+</sup></sup>/ER-Cre<sup>+/−</sup> or IGF-1R<sup>loxP<sup>+/+</sup></sup>/ER-Cre<sup>−/−</sup> mice were injected (i.p.) with tamoxifen (1 or 3 mg per mice) for 7 days. Vena cavae from these mice were used to create a vein graft in carotid arteries of tamoxifen-treated mice with the same transgene. IGF-1R expression in the vein graft after 4 weeks was analyzed by RT-PCR (B) and Western blot (C). D, Knockout of IGF-1R suppresses neointima formation in vein graft. Veins from the graft were stained with HE (upper left panel, ×10). Immunostaining of SMA, IGF-1R, PDGFR, EGFR, pAKT, p27<sup>kip1</sup> were performed in tamoxifen-treated vein grafts (magnification, ×40). Data are shown as representative results from 6 mice in each group. E, The lumen and neointimal areas were quantified using AxiowVision and NIH Image J software. F, Lumen and media ratio were calculated from tamoxifen-treated mice (∗P<0.01, compared with control, n=6).
tion of IGF-1R at early phase (5 to 15 minutes) was abolished (Figure 2D). This result indicates that c-Src activation leads to ligand-independent tyrosine phosphorylation of IGF-1R. Mechanical activation of c-Src also leads to phosphorylation of other receptor tyrosine kinases such as VEGFR2, PDGFR β, and EGFR. In fact, there is evidence that IGF-1R is tyrosine phosphorylated and enzymatically activated by Src tyrosine kinase.17 Notably, mechanical stretch not only activates IGF-1R via c-Src kinase but also leads to autocrine production of IGF-1, which produces a late-phase tyrosine phosphorylation of IGF-1R and IRS-1. These responses explain why blocking IGF-1 responses with a neutralizing IGF-1 antibody will abolish the late phase IGF-1R and IRS-1 activation (Figure 2B).

The importance of our finding that activation of IGF-1/IGF-1R signaling is a response to mechanical stretch is that prolonged activation of IRS-1–associated PI3K activity. It has been shown that prolonged activation of PI3K plays an important role in cell proliferation, migration, and survival.25 For example, activated PI3K stimulates AKT or serum glucocorticoid regulated kinase, SGK-1, to increase cell cycle progression. This response is attributable to expression of cell cycle–specific genes such as cyclin D or posttranslational modification of cell cycle signal proteins (eg, phosphorylation of GSK3β, FOXO, or p27(kip1)).

In summary, mechanical stretch activation of IGF-1R is essential for the accumulation of VSMCs in the vein graft, and this pathway contributes to the formation of neointima and the failure of hemodialysis accesses or coronary bypass grafts.

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Disclosures
None.

References
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