largely focused on microRNAs that regulate expression of genes encoding specific disease-causing proteins, such as amyloid precursor protein in Alzheimer's disease and synuclein in Parkinson's disease, or cellular systems related to neurodegeneration, such as neuroinflammation. A strength of the current study is the identification of AMPAR subunits as the relevant targets of miR-124 in *CHMP2B*^{Intron5} mice by providing evidence that manipulating AMPARs can correct sociability deficits.

The observation that changing AMPAR composition in one brain region can alter social behavior is fascinating, though not without precedent. Social dominance is also modulated by AMPAR composition in the mPFC: increasing GluA4 expression increases social dominance, whereas disrupting GluA4 trafficking to the synapse reduces social dominance[11]. These studies add to a growing literature implicating postsynaptic glutamatergic mechanisms in regulating social behavior[12].

Gascon *et al.*[6] showed decreased calcium permeability in mPFC AMPARs in *CHMP2B*^{Intron5} mice corresponding to an increase in GluA2 subunits. This is somewhat surprising because increased calcium permeability of AMPARs has been associated with amyotrophic lateral sclerosis (ALS)[13], a neurodegenerative condition closely related to FTD. Furthermore, most AMPARs in the brain are considered to contain GluA2, making them calcium impermeable at baseline. However, AMPAR subunit composition varies by CNS region. Whereas spinal motor neurons affected in ALS normally express mostly calcium-impermeable AMPARs and are sensitive to excitotoxic effects of increased calcium permeability with loss of normal GluA2, the current study suggests that mPFC neurons affected in FTD normally express some calcium-permeable (GluA2-lacking) AMPARs and are sensitive to dysfunction caused by insufficient calcium signaling when GluA2 expression increases.

In the last few years, several new FTD-associated genes have been identified, many of which had not previously been studied in the nervous system. Many new mouse models expressing mutant forms of these genes develop social deficits and other changes consistent with the behavioral symptoms seen in FTD[14]. Studies such as this report from Gascon *et al.*[6] are beginning to dissect the neurobiological basis of FTD symptoms, in both sporadic and inherited cases, and are identifying new targets for translation into therapeutic strategies.

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Antiangiogenic VEGF-A in peripheral artery disease

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Vascular endothelial growth factor A (VEGF-A) is a potent proangiogenic cytokine elevated in patients with peripheral artery disease (PAD). A new study links impaired vascular regrowth in PAD to increased expression of an antiangiogenic splice variant of VEGF-A.

PAD results from blockade of arteries, most often in the legs, due to atherosclerotic lesions. Patients with PAD experience severe pain due to limb ischemia and are at elevated risk for myocardial infarction, stroke and amputation[1]. The risk of developing PAD is increased in people with diabetes, metabolic disease and obesity, and these risk factors also correlate with decreased ability to provide alternate conduits for blood flow. Currently available treatments are limited and often have minimal efficacy on tissue revascularization, and molecular mechanisms regulating revascularization in PAD are poorly understood. In this issue of *Nature Medicine*, Kikuchi *et al.*[2] show that an antiangiogenic isoform of VEGF, VEGF-A$_{165}$b, is elevated in patients with PAD and prevents revascularization in a preclinical model of ischemia, suggesting new targets for relief of PAD symptoms.

In patients with PAD, blood flow is hindered, often resulting in tissue ischemia (**Fig. 1**). Collateral vessel remodeling and/or angiogenesis can mitigate ischemia by providing alternate conduits for blood flow. Collateral vessels bypass the capillary bed and connect arteries to each other, and angiogenesis promotes new vessel growth from preexisting vessels. A major proangiogenic signal that regulates vessel growth and collateral remodeling is VEGF-A. VEGF-A RNA is alternatively spliced to yield protein isoforms of different sizes, and the VEGF-A$_{165}$ isoform activates angiogenesis and collateral vessel remodeling. Despite its proangiogenic function, gene therapy based on VEGF-A has had surprisingly limited success in clinical trials for PAD[3], and patients with PAD have impaired revascularization despite elevated endogenous levels of circulating VEGF-A$_{165}$ (ref. 4). Further alternative splicing of VEGF-A$_{165}$ RNA gives rise to two isoforms in humans, VEGF-A$_{165}$a and VEGF-A$_{165}$b. VEGF-A$_{165}$a is a potent survival cue and mitogen for endothelial cells and hence stimulates vascular growth, whereas VEGF-A$_{165}$b is an antiangiogenic splice variant that inhibits VEGF-A$_{165}$a binding to its receptor[5]. VEGF-A$_{165}$b has been positively associated with inflammatory myopathies and sclerosis and negatively associated with cancer[6,7]. Levels of VEGF-A$_{165}$b are elevated in muscle of patients with PAD following exercise therapy[8]; however, its link to ischemic diseases such as PAD is poorly understood.

In this study, Kikuchi *et al.*[2] investigated why patients with PAD display insufficient revascularization of ischemic tissue despite elevated serum levels of total VEGF-A$_{165}$. They found high circulating levels of antiangiogenic VEGF-A$_{165}$b in patients with PAD and low levels of proangiogenic VEGF-A$_{165}$a. Inflammatory cells contribute to the progression of PAD and are also a known source of

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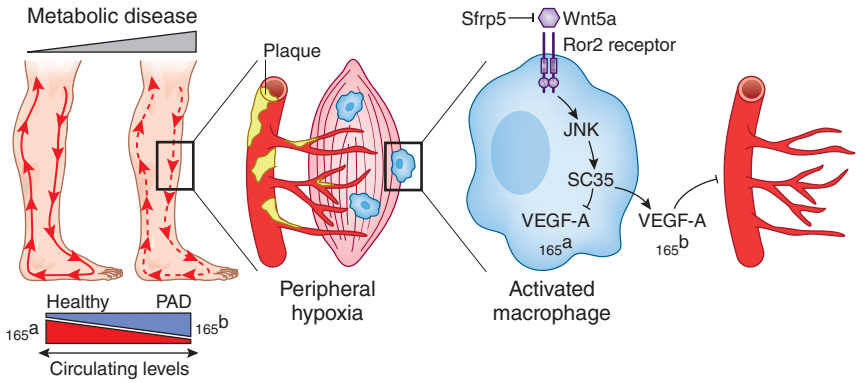

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Figure 1 Patients with PAD have decreased blood flow in leg arteries resulting from atherosclerotic plaques. Surrounding muscle becomes ischemic, which results in the recruitment of activated macrophages. Kikuchi *et al.*[2] have now shown that an antiangiogenic isoform of VEGF-A, VEGF-A_{165}b, is upregulated in PAD, whereas the proangiogenic isoform VEGF-A_{165}a is downregulated. Macrophages bind Wnt5a via the Ror2 receptor, leading to JNK activation and upregulation of the RNA splicing factor SC35. SC35 splices VEGF-A RNA to produce the antiangiogenic VEGF-A_{165}b isoform over the proangiogenic VEGF-A_{165}a isoform, preventing revascularization to mitigate effects of PAD. Risk for PAD is linked to metabolic disease. Sfrp5 is an inhibitor of Wnt5a signaling that protects against obesity-induced inflammation. The authors link loss of Sfrp5 to increased vascularization and so provide a connection between the two conditions.

VEGF-A_{165}. Consistent with this, monocytes isolated from patients with PAD had elevated levels of VEGF-A_{165}b RNA and low levels of VEGF-A_{165}a RNA compared to healthy individuals, suggesting that monocytes are a major source of the shift in VEGF-A_{165} isoform levels in these patients.

Wnt5a is a proinflammatory protein found at elevated levels in patients with metabolic disease[9]. Noncanonical Wnt5a signaling is linked to impaired angiogenesis in mice by promoting alternative splicing of VEGF receptor-1 (ref. 10), so Kikuchi *et al.*[2] also investigated the role of Wnt5a in VEGFA_{165}b isoform production in PAD. Wnt5a expression was increased in peripheral blood mononuclear cells of patients with PAD, and this increase correlated with elevated serum levels of VEGF-A_{165}b. Both parameters individually correlated with disease severity in PAD.

The muscle ischemia of PAD can be modeled in mice via femoral artery ligation that leads to hind limb ischemia. Wild-type (WT) mice had elevated Wnt5a expression in ischemic muscle after ligation, and staining revealed that Wnt5a co-localized with a macrophage marker. Mice selectively overexpressing Wnt5a in myeloid cells (Wnt5a GOF) were used to analyze the effects of Wnt5a on tissue revascularization in ischemic tissue. WT and Wnt5a GOF mice both had elevated expression of total VEGF-A after femoral artery ligation, but only Wnt5a GOF mice showed a prolonged impairment in revascularization. Prior to this study, it was not clear that VEGF-A_{164}b, the murine form of VEGF-A_{165}b, was produced in mice. The use of a murine VEGF-A_{164}b isoform–specific antibody developed for the study showed that VEGF-A_{164}a was predominant in WT mice, whereas VEGF-A_{164}b was predominant in Wnt5a GOF mice in response to ischemia. Adenovirus-mediated overexpression of VEGF-A_{164}b reduced revascularization in WT ischemic mice, whereas injection of VEGF-A_{164}b–neutralizing antibodies in Wnt5aGOF mice improved revascularization of ischemic tissue. These results indicate that macrophage-derived Wnt5a leads to VEGF-A_{164}b production that inhibits revascularization in mice with hind limb ischemia.

Clinically, metabolic disease is a risk factor for PAD. On the basis of findings that the Wnt5a–VEGF-A_{165}b axis is antiangiogenic in mice, the function of VEGF-A_{164}b in metabolic disease–induced vascularization impairments was also investigated. Mice with diet-induced obesity or leptin deficiency (*ob/ob*, diabetes-like syndrome) had increased Wnt5a levels and macrophage infiltration of ischemic limbs, which correlated with elevated levels of VEGF-A_{164}b and impaired revascularization. Sfrp5 is an anti-inflammatory protein that exerts beneficial effects on metabolic dysfunction, in part by inhibiting Wnt5a signaling[11]. Kikuchi *et al.*[2] showed that mice lacking Sfrp5 have elevated VEGF-A_{164}b levels, increased macrophage infiltration and impaired revascularization of hind limb ischemia. Importantly, limb revascularization was rescued by injection of VEGF-A_{164}b–neutralizing antibodies into obese or Sfrp5-deficient mice. Collectively, these findings show that Wnt5a levels correlate with VEGF-A_{164}b levels and vascular pathology *in vivo* in the context of metabolic dysfunction, and they suggest that Wnt5a blocks ischemic revascularization via VEGF-A_{165}b production downstream of Sfrp5. The results also provide proof of concept that blockade of VEGF-A_{165}b has therapeutic potential for patients with PAD.

Finally, the authors carried out *in vitro* experiments to characterize a mechanism by which Wnt5a signaling leads to VEGF-A_{165}b isoform production in macrophages[2]. These experiments revealed that alternative splicing to produce VEGF-A_{165}b is regulated by SC35, an RNA-splicing factor that regulates VEGF-A_{165} splicing in other cell types[12]. Wnt5a signaled through the Ror2/Fz receptor to activate the c-Jun N-terminal kinase (JNK) pathway and upregulated spicing factor SC35 (**Fig. 1**). This mechanistic understanding of Wnt5a-mediated VEGF-A_{165} splicing in macrophages may help the development of new therapies for PAD.

One caveat of this study is that the mouse model used induces acute, localized ischemia in the hind limb, whereas ischemia during human PAD is chronic and widespread. Thus, an open question is whether blockade of VEGF-A_{165}b would be sufficient to improve vascularization in patients with PAD. Additional questions are also raised by this study. For example, does SC35-mediated production of VEGF-A_{165}b occur in healthy individuals, or is this pathway unique to pathological conditions? Furthermore, SC35, a splicing factor with many targets that has been associated with splicing clusters in the nucleus[13], may regulate a network of pathways that impact vascular function. Indeed, mutation of SC35 has been linked to myelodysplasia via aberrant splicing of numerous RNAs[14]. Thus, therapeutic modulation of SC35 in PAD may not be restricted to modulation of VEGF-A_{165}b and may provide a more comprehensive rescue of vascular function. Other growth factor signaling pathways also activate distinct splicing pathways that regulate VEGF-A_{165}b production[15], perhaps in a cell type–specific manner, so targeting splicing machinery as a clinical strategy will probably require further elucidation of the pathway.

Although the antiangiogenic role of the VEGF-A_{165}b isoform had been reported previously, these new findings link VEGF-A_{165}b to PAD directly and highlight its abnormal regulation in metabolic disease via Sfrp5 and Wnt5a. This work improves our understanding of the consequences of PAD-associated vascular deficiency by showing that VEGF-A_{165}b prevents ischemic revascularization. It also provides an explanation for the paradox that vessel growth is impaired despite high circulating levels of VEGF-A_{165} in patients with PAD, and it identifies a cellular source and mechanism of VEGF-A_{165}b production in preclinical models of PAD. Although much

work remains to precisely define the role of VEGF-A_{165}b in PAD and other ischemic diseases, this study by Kikuchi *et al.*[2] enhances our understanding of mechanisms underlying this devastating disease and suggests new therapeutic targets.

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