RESEARCH PAPER

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TRF2 acts as a transcriptional regulator in tumor angiogenesis

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Abbreviations: HS3ST4, heparan sulfate (glucosamine) 3-O-sulfotransferase 4; HIF1A, hypoxia-inducible factor 1 α subunit; LEC, lung endothelial cells; PDGFRB, platelet-derived growth factor receptor β; TRF2, telomeric repeat-binding factor 2; TEC, tumor endothelial cell; VEGF, vascular endothelial growth factor; WT1, Wilms' tumor suppressor 1.

We recently showed that telomeric repeat-binding factor 2 (TRF2) regulates gene expression to promote angiogenesis. We found that TRF2 is highly expressed in tumor vessels and transcriptionally activates platelet-derived growth factor receptor β to promote endothelial cell angiogenic properties independently of its function in telomere protection. This work identifies TRF2 as a promising dual target for cancer therapy.

Angiogenesis is important for cancer progression. Inhibition of tumor angiogenesis is widely accepted as a valuable therapeutic option for cancer treatment and a large number of cancer patients benefit from treatment with inhibitors of the angiogenic vascular endothelial growth factor (VEGF). However, different tumor types show unique features in their vasculature depending on tissue specificity and the expression pattern of pro- and anti-angiogenic molecules and their cognate receptors. Thus, it is not surprising that, in addition to positive antitumor effects, increased invasion and metastasis have also been noted in divergent experimental models targeting VEGF.[1] These observations support the notion that other proangiogenic factors play a role in sustained tumor angiogenesis and progression.

We previously found that the Wilms' tumor suppressor WT1 is highly expressed in vessels of human tumors but not in healthy adjacent tissues, and is required for angiogenesis.[2] Furthermore, WT1 is implicated in vessel formation during development and in pathophysiological conditions such as myocardial ischemia as a transcriptional target of hypoxia-inducible factor 1 α (HIF1A).[3,4] *WT1* encodes a zinc-finger transcription factor with crucial functions in development and was originally identified as a tumor suppressor based on its mutational inactivation in Wilms' tumor.[5] However, because of its overexpression in a variety of human cancers, it is now considered a potential oncogene.

Like WT1, expression of the telomeric repeat-binding factor 2 (TRF2, also known as TERF2), which plays a central role in telomere capping, is frequently increased in human tumors.[6] Consistent with a potent oncogenic role of a high level of TRF2, its downregulation in cancer cells reduces tumorigenicity whereas its overexpression favors oncogenesis.[7] We showed that the high level of TRF2 found in cancer cells positively regulates the expression of *HS3ST4* encoding heparan sulfate (glucosamine) 3-O-sulphotransferase 4, which is involved in the recruitment of NK-cells, thus revealing that TRF2 upregulation can have oncogenic properties through cell non-autonomous mechanisms.[7]

In our recent work, we identified TRF2 as a transcriptional regulator in tumor endothelial cells (**Fig. 1**). We found that TRF2 was highly expressed in the tumor vasculature of different human cancer types, but not in the vessels of healthy tissues. Using *ex vivo* approaches, we demonstrated that tumor-derived endothelial cells (TECs) expressed higher levels of Trf2 than endothelial cells from healthy lung tissues (LECs) and exhibited a higher angiogenic potential. We further showed that upregulation of *Trf2* in the tumor endothelium is the result of its transcriptional activation by WT1.[8] In agreement with an important angiogenic role of Trf2 overexpression in TECs, depletion of Trf2 in these cells led to loss of their angiogenic properties, and overexpression of Trf2 in LECs conferred on them the high angiogenic potential of TECs.[8] Overall, these findings suggest that TRF2 might play a role in vascular development and in cases of ischemia, as has been shown for WT1.[3,4]

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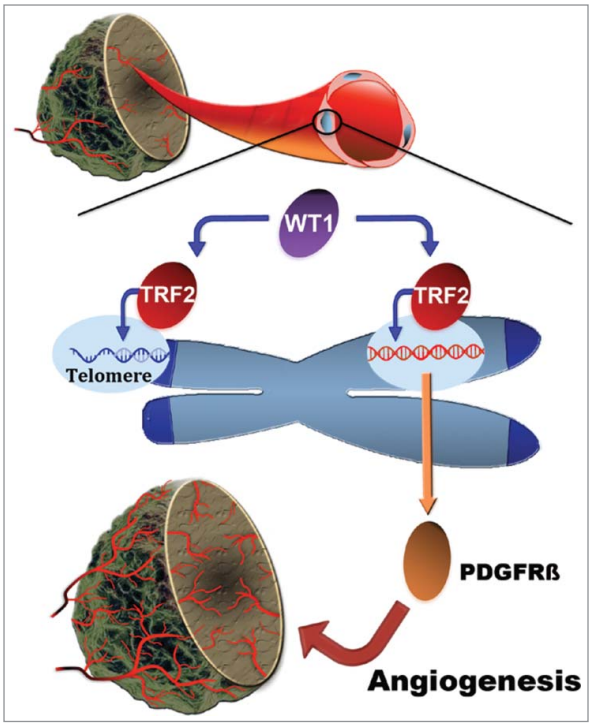

Figure 1. Telomeric repeat-binding factor 2 (TRF2) regulates angiogenesis by acting as a transcription factor independent of its function in telomere protection. TRF2 is overexpressed in many cancer cell types. Furthermore, Trf2 expression is upregulated in tumor-derived endothelial cells, most likely because *TRF2* is a transcriptional target of the Wilms' tumor suppressor 1 (WT1), which is highly expressed in the tumor vasculature. In tumor endothelial cells, Trf2 binds and transactivates the platelet-derived growth factor receptor β (*Pdgfr*β) promoter, which stimulates angiogenesis. This represents a non-canonical function of TRF2. Overall, by activating tumor angiogenesis and cancer cell proliferation, TRF2 emerges as a valuable multi-hit target for cancer therapy.

Next, we investigated how a high TRF2 level boosted the angiogenic properties of TECs. First, we did not observe telomere uncapping and initiation of the DNA damage response (DDR) upon modulation of TRF2 levels, indicating that the angiogenic function of TRF2 in endothelial cells is uncoupled from its role in telomere capping. Second, by screening for angiogenic targets of TRF2, we found that TRF2 increases the expression of platelet-derived growth factor receptor β (PDGFRB).[8] PDGFRB promotes angiogenesis and is frequently upregulated in cancer endothelia. Autocrine growth stimulation of tumor endothelial cells through PDGFRB that is evoked by interaction with cancer cells has been postulated, underlining the diversity of proangiogenic mechanisms in tumor angiogenesis and the resulting difficulties in establishing adequate antiangiogenic therapies for cancer treatment.[9] Third, using different approaches we demonstrated that TRF2 is a direct transcriptional activator of *PDGFRB* that is capable of binding and activating the *PDGFRB* promoter. Overexpression of *PDGFRB* could rescue the inhibition of endothelial cell proliferation caused by depletion of TRF2, but only partially rescued the effects on the migratory potential of these cells.[8] This indicates that TRF2 might also activate factors other than PDGFRB that are implicated in endothelial cell migration.

The fact that telomere factors such as TRF2 bind extra-telomeric sites to regulate transcription is not unprecedented (reviewed in[10]). Our finding that TRF2 binds and activates the *PDGFRB* promoter should facilitate dissection of the mechanism by which TRF2 acts as a transcriptional regulator and consequently will have strong implications for basic and biomedical research.

Taken together, these findings reveal a novel mechanism by which TRF2 transcriptionally controls tumor angiogenesis independent of its role in telomere protection. They further suggest that pathways controlling replicative potential (telomere) and those controlling energy supply (angiogenesis) co-evolved for a better control of tissue homeostasis and renewal. They also point to TRF2 as a novel multi-hit target in cancer therapy, as it is implicated in both the proliferation of cancer cells through cell autonomous and non-autonomous mechanisms and in the angiogenic properties of TECs. Thus, compared to classic antiangiogenic therapies, the identification of molecules that target TRF2 might be a major advance for cancer treatment through hitting 2 birds with one stone.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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