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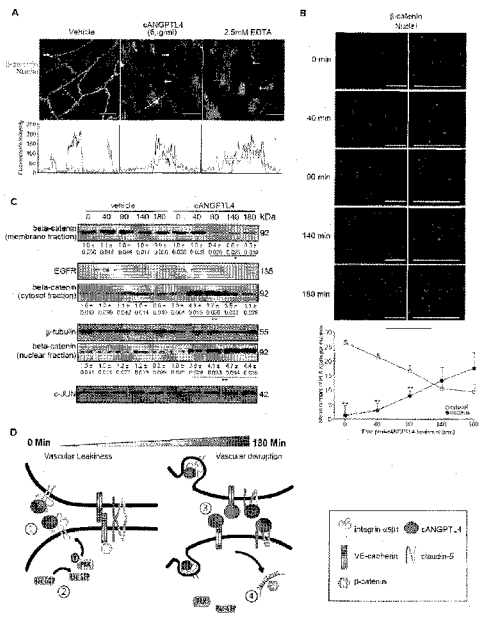
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

[Continued on next page]

(54) **Title**: ANGIOPOIETIN-LIKE 4 AND ITS USE IN MODULATING CELL LEAKINESS

FIGURE 6



(57) **Abstract**: Vascular disruption induced by interactions between tumor-secreted permeability factors and adhesive proteins on endothelial cells facilitates metastasis. The role of tumor secreted angiopoietin-like 4 (cANGPTL4) in vascular leakiness and metastasis is controversial due to the lack of understanding of how cANGPTL4 modulates vascular integrity. Here, we show that cANGPTL4 instigated the disruption of endothelial continuity by directly interacting with three novel binding partners, integrin $\alpha5\beta1$, VEcadherin and claudin-5, in a temporally sequential manner, thus facilitating metastasis. We showed that cANGPTL4 binds and activates integrin $\alpha5\beta1$-mediated Rac1/PAK signaling to weaken cell-cell contacts. cANGPTL4 subsequently associated with and declustered VE-cadherin and claudin-5, leading to endothelial disruption. Interfering with the formation of these cANGPTL4 complexes delayed vascular disruption. In vivo vascular permeability and metastatic assays performed using *ANGPTL4*-knockout and wild-type mice injected with either control or ANGPTL4-knockdown tumors confirmed that cANGPTL4 induced vascular leakiness and facilitated lung metastasis in mice. Thus, our findings elucidate how cANGPTL4 induces endothelial disruption. Our findings have direct implications for targeting cANGPTL4 to treat cancer and other vascular pathologies.

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