Oral Oncology 47 (2011) 371–375

Contents lists available at ScienceDirect

Oral Oncology

journal homepage: www.elsevier.com/locate/oraloncology

Angiopoietin-like 4: A novel molecular hallmark in oral Kaposi's sarcoma

Jiadi Hu [a], Bruno C. Jham [a], Tao Ma [a], Eitan R. Friedman [a], Leticia Ferreira [d], John M. Wright [d], Brent Accurso [e], Carl M. Allen [e], John R. Basile [a,c], Silvia Montaner [a,b,c,*]

[a] Department of Oncology and Diagnostic Sciences, School of Dentistry, University of Maryland, Baltimore, MD 21201, USA
[b] Department of Pathology, School of Medicine, University of Maryland, Baltimore, MD 21201, USA
[c] Greenebaum Cancer Center, University of Maryland, Baltimore, MD 21201, USA
[d] Department of Diagnostic Sciences, Texas A&M Health Science Center, Baylor College of Dentistry, Dallas, TX 75246, USA
[e] College of Dentistry, Division of Oral and Maxillofacial Surgery, Pathology, and Anesthesiology, The Ohio State University, Columbus, OH 43210, USA

ARTICLE INFO

Article history:
Received 13 December 2010
Received in revised form 11 February 2011
Accepted 19 February 2011
Available online 21 March 2011

Keywords:
Oral cavity
Kaposi's sarcoma
Kaposi's sarcoma associated herpesvirus, KSHV
Human herpesvirus-8
HHV-8
G protein-coupled receptor
vGPCR
Angiopoietin
Angiopoietin-like 4
Vascular endothelial growth factor
Angiogenesis
Vascular permeability

SUMMARY

Kaposi's sarcoma (KS) remains among the most common causes of oral cancer in HIV-infected individuals. Infection with the KS-associated herpesvirus (KSHV/HHV8) is a necessary event for disease development. Emerging evidence suggests that KSHV infects vascular endothelial (or endothelial progenitor) cells promoting the formation of the KS tumor (or spindle) cell. These cells elaborate angiogenic growth factors and cytokines that promote the dysregulated angiogenesis and profuse edema that characterizes this unusual vascular tumor. Central among these secreted factors is the potent endothelial cell mitogen, vascular endothelial growth factor (VEGF). Indeed, VEGF has proven to be a key player in KSHV pathogenesis and is a molecular hallmark of KS lesions. We have recently shown that a second angiogenic factor, Angiopoietin-like 4 (ANGPTL4), may also play a critical role in KS development. Here we demonstrate that ANGPTL4 is upregulated both directly and indirectly by the KSHV oncogene, vGPCR. We further show that ANGPTL4 is a molecular hallmark of oral KS lesions. Indeed, expression of this protein was observed in more tumor cells and in more biopsies specimens than expression of VEGF (23/25 or 92% vs. 19/25 or 76%, respectively) in oral KS. These surprising results support a key role for ANGPTL4 in Kaposi's sarcomagenesis and further suggest that this angiogenic factor may provide a novel diagnostic and therapeutic marker for oral KS patients.

Introduction

KS is a multifocal vascular neoplasm that often affects the oral cavity in immunosuppressed patients.[1] First described as a skin cancer in older men of Jewish or Mediterranean ancestry, a dramatic change in the epidemiology and clinical course of KS occurred with the emergence of the acquired immune deficiency syndrome (AIDS).[2] Today, KS remains as one of the most common malignancies affecting HIV-infected individuals and is the most frequent cancer among children and adult men in countries of sub-Saharan Africa.[2] Unfortunately, clinical management of KS continues to be a challenge.

A scientific leap in our understanding of the pathogenesis of KS was made possible with the identification of a novel human herpesvirus, HHV8, named Kaposi's sarcoma associated herpesvirus (KSHV), as the etiological agent for this tumor.[3] Subsequent work from several groups suggests that endothelial cell infection with KSHV is indeed a prerequisite for KS development, and results in the formation of the KS tumor (or spindle) cell. These KS spindle cells are the driving force of KS lesion, elaborating angiogenic growth factors and cytokines that promote the formation of this vascular tumor.[2]

Emerging evidence supports a key role for a viral protein, the KSHV G protein-coupled receptor (vGPCR), in the initiation and promotion of KS.[2] vGPCR is a member of the family of CXC chemokine GPCRs, with closest homology to CXCR2, but with ligand-independent (constitutive) activity. Endothelial cells expressing vGPCR elaborate angiogenic growth factors and cytokines that have been suggested to promote tumor formation through a unique paracrine mechanism.[2] Indeed, transgenic mice that express vGPCR manifest dermal angioproliferative lesions that closely resemble those seen

* Corresponding author at: Department of Oncology and Diagnostic Sciences, School of Dentistry, University of Maryland, Baltimore, 650 W. Baltimore Street, 7th North, Rm 7263, Baltimore, MD 21201, USA. Tel.: +1 410 706 7944; fax: +1 410 706 0519.
E-mail address: smontaner@umaryland.edu (S. Montaner).

1368-8375/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.oraloncology.2011.02.018

in KS.[4–6] These observations have prompted intense investigation into identifying the molecular mechanism(s) whereby vGPCR could play a role in Kaposi's sarcomagenesis.

Initial work on the contribution of vGPCR to KS development appropriately centered on the upregulation by this viral receptor of the potent endothelial cell mitogen, VEGF, a key player in KSHV pathogenesis.[7,8] However, we recently identified a novel angiogenic factor, ANGPTL4, which also appears to play an essential role in vGPCR tumorigenesis, promoting angiogenesis and vascular permeability.[9] Here we set out to examine the prevalence of ANGPTL4 upregulation in oral KS lesions.

Materials and methods

Cell lines and reagents

pCEFL AU5 vGPCR, pCEFL AU5 GFP, pBIG AU5 vGPCR and pCEFL Tet REV TA have been previously described.[5,9] HMEC1s were obtained from the CDCs (Atlanta, GA) and grown as described elsewhere.[9] Cells were transfected with Polyfect (Qiagen). Conditioned media was prepared as previously described.[9] Recombinant proteins were purchased from Pepro Tech. Concentrations used are: IL-8 (50 ng/ml), GROα (50 ng/ml), PDGF (25 ng/ml), IL-1β (10 ng/ml), IL-10 (25 ng/ml), IL-6 (2 ng/ml), TNFα (25 ng/ml), IP-10 (50 ng/ml), SDF1α (80 ng/ml), VEGF (50 ng/ml), and ANGPTL4 (5 μg/ml).

Additional information can be found in the Supplemental materials and methods.

Results

KS is a vascular tumor promoted by KSHV infection and the resultant expression of different viral genes and microRNAs.[2] Work from several labs has supported a key role for dysregulated expression of the KSHV-encoded GPCR (vGPCR) in the promotion of KS.[2] Transgenic mice expressing this viral receptor in endothelial cells manifest vascular tumors (vGPCR tumors) histologically similar to human KS, with expression of vGPCR limited to a few cells, suggestive of a paracrine mechanism for vGPCR tumorigenesis.[5] Indeed, expression of vGPCR in cultured endothelial cells stimulates the release of angiogenic growth factors and pro-inflammatory chemokines and cytokines.[7,8,10–13] Of interest, expression of vGPCR in immortalized human dermal microvascular endothelial cells (HMEC1s) led to an increase in the mRNA levels of a novel hypoxia-regulated factor, *Angiopoietin like-4*, a member of the family of Angiopoietin-like proteins (ANGPTLs), which has been shown to play an important role in the control of angiogenesis[9,14] (Fig. 1A). Indeed, induction of vGPCR expression in HMEC1s using a tetracy-

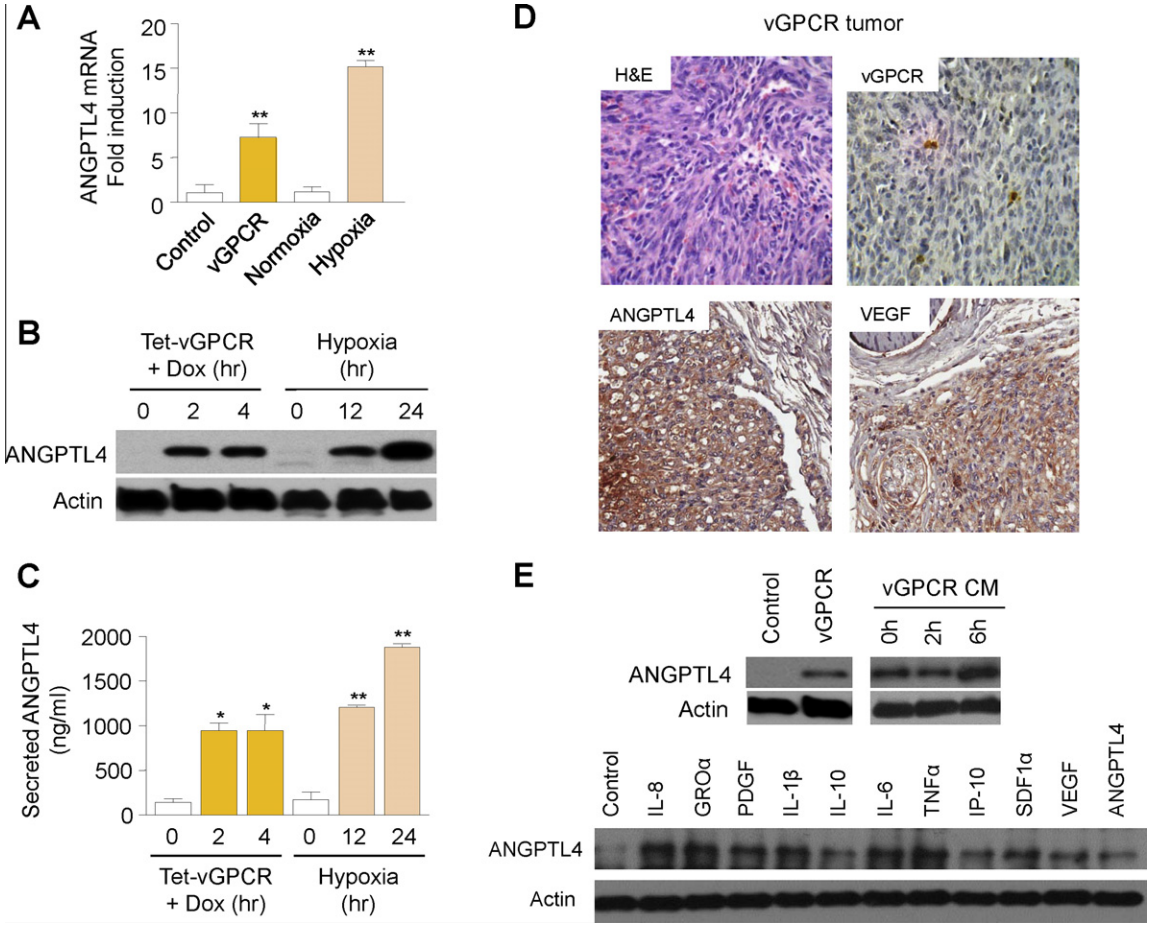

Fig. 1 Direct and paracrine upregulation of ANGPTL4 by vGPCR. (A) *angptl4* mRNA levels (qRT-PCR), upon transfection of pCEFL AU5 vGPCR (vGPCR) or pCEFL AU5 GFP (Control) in HMEC1. Induction of *angptl4* mRNA by hypoxia (1% O_2; 24 h) was used as a control. (B and C) Cellular ANGPTL4 (WB) (B) and secreted ANGPTL4 (ELISA) (C) of HMEC1 transfected with pCEFL Tet REV TA and pBIG AU5 vGPCR (Tet–vGPCR). Cells were left untreated or treated with (1 μg/ml) Dox for 2 or 4 h. Induction of ANGPTL4 expression by hypoxia (1% O_2; 12 or 24 h) was used as a control. (D) Representative H&E staining and immunohistochemical detection of (AU5) vGPCR-expressing cells as well as ANGPTL4 and VEGF expression in murine vGPCR tumors. (E) Upregulation in HMEC1 of ANGPTL4 upon transfection of pCEFL AU5 vGPCR (vGPCR) or pCEFL AU5 GFP (Control), treatment with conditioned media of vGPCR-expressing cells (vGPCR CM), or exposure to individual recombinant factors present in vGPCR conditioned media.