Experimental and Molecular Pathology 97 (2014) 148–153

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

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

Angiogenic growth factor expression in benign and malignant vascular tumours

Robin J. Young [a,b,*], Malee Fernando [c], David Hughes [c], Nicola J. Brown [b], Penella J. Woll [a]

[a] Academic Unit of Clinical Oncology, Weston Park Hospital, Whitham Rd, Sheffield S10 2SJ, UK
[b] Academic Unit of Surgical Oncology, Medical School, Beech Hill Rd, University of Sheffield, Sheffield S10 2RX, UK
[c] Department of Histopathology, Sheffield Teaching Hospitals NHS Foundation Trust, Glossop Rd, Sheffield S10 2JF, UK

ARTICLE INFO

Article history:
Received 24 June 2014
Available online 28 June 2014

Keywords:
Angiosarcoma
Angiogenesis
VEGF

ABSTRACT

Angiosarcomas are rare malignant vascular tumours. Angiosarcoma expression of vascular endothelial growth factor (VEGF) has previously been reported, but angiosarcoma expression of other angiogenic growth factors has not been systematically studied. Non-VEGF angiogenic growth factors are a potential mechanism of resistance to VEGF-targeted therapy, but they also represent potential therapeutic targets.

Immunohistochemistry analysis evaluated the expression of 13 angiogenic growth factors and receptors in 27 separate benign and malignant archived human vascular tumour samples. The expression of 55 angiogenesis-related proteins was subsequently profiled in five fresh human angiosarcoma tumour samples using antibody arrays.

Angiosarcomas expressed a variety of angiogenic growth factors. Significantly higher levels of Notch1 were detected compared with benign haemangiomas ($p = 0.033$), but lower levels of basic fibroblast growth factor (bFGF) compared to benign haemangiomas ($p = 0.07$) and inflammatory vascular lesions ($p = 0.009$). Vascular tumour expression of FGF receptor (FGFR)-1 correlated with angiopoietin (Ang)-2, Tie2, hepatocyte growth factor (HGF) and Notch1 expression ($p = 0.001$, $p = 0.001$, $p < 0.001$ and $p < 0.001$ respectively). Notch1 also correlated with Tie2 expression ($p = 0.004$).

In conclusion, angiosarcomas express multiple angiogenic growth factors. Treatments could be targeted at individual angiogenic growth factors. However, our findings provide a rationale for combination therapy, or for treatments that target common downstream signalling intermediaries, such as Akt, mTOR or ERK.

© 2014 Elsevier Inc. All rights reserved.

1. Introduction

Vascular tumours encompass a spectrum of disease ranging from indolent haemangiomas to aggressive angiosarcomas. Haemangiomas are common benign lesions, which consist of a profusion of small blood vessels that form enlarged thin walled vascular spaces lined by quiescent flattened endothelial cells (Weiss and Goldblum, 2001a). Pyogenic granulomas, also known as lobular capillary haemangiomas, represent a distinct subtype of haemangioma. They are rapidly growing, highly cellular lesions of proliferating neoplastic endothelial cells packed in a lobular pattern, often with a florid inflammatory infiltrate (Weiss and Goldblum, 2001a). In contrast to haemangiomas, angiosarcomas are rare, extremely aggressive vascular tumours. The malignant cells of well-differentiated angiosarcomas form irregular vascular channels, however poorly-differentiated lesions demonstrate minimal or no discernible vascular architecture (Weiss and Goldblum, 2001b).

Angiogenesis, the process of new blood vessel formation from the existing vasculature, is tightly regulated by a complex network of pro and anti-angiogenic growth factors (Herbert and Stainier, 2011). Angiogenesis is critical for tumour growth and metastasis (Hanahan and Weinberg, 2000), but it is dysregulated in malignancy, which results in tumour vasculature with abnormal form and function. There is intense interest in the role of angiogenesis in the pathobiology of vascular tumours. To-date, studies of vascular tumours have focused on vascular endothelial growth factor (VEGF) expression, which is a potent pro-angiogenic stimulant. Suppressed expression of the VEGF decoy receptor VEGFR1 in tumour endothelial cells of infantile haemangioma enhances VEGF signalling through VEGFR2, which promotes tumour endothelial cell proliferation (Jinnin et al., 2008). Human angiosarcoma tumour endothelial cells have been reported to over-express VEGF and VEGFR1-3 (Itakura et al., 2008). However the relationship between receptor expression and angiosarcoma tumour biology is uncertain. Notably, phase II clinical trials of VEGF-targeted agents for the treatment of angiosarcoma have yielded disappointing results with response rates of only 15% (George et al., 2009; Maki et al., 2009; Ray-Coquard et al., 2012).

* Corresponding author at: Academic Unit of Clinical Oncology, Weston Park Hospital, Whitham Rd, Sheffield S10 2SJ, UK.
E-mail addresses: r.j.young@sheffield.ac.uk (R.J. Young), malee.fernando@sth.nhs.uk (M. Fernando), david.hughes@sth.nhs.uk (D. Hughes), n.j.brown@sheffield.ac.uk (N.J. Brown), p.j.woll@sheffield.ac.uk (P.J. Woll).

http://dx.doi.org/10.1016/j.yexmp.2014.06.010
0014-4800/© 2014 Elsevier Inc. All rights reserved.

Other known angiogenic growth factors and their associated receptors include angiopoietins (Ang)/tyrosine kinase with immunoglobulin-like and EGF-like domains (Tie), fibroblast growth factors (FGF)/FGF receptors (FGFR), hepatocyte growth factor (HGF)/MET, and delta-like ligand (DLL)/Notch. Compensatory tumour expression of non-VEGF angiogenic growth factors may explain the poor response of angiosarcomas to VEGF-targeted agents, and themselves represent potential therapeutic targets using agents that are currently in clinical development, such as trebananib, an angiopoietin neutralising peptibody, or crizotinib, a Met tyrosine kinase inhibitor. Until now, the expression of non-VEGF angiogenic growth factors in vascular tumours has not been systematically studied. Here, we report the expression of 13 angiogenic growth factors and receptors (VEGF, VEGFR1, VEGFR2, neuropilin (NRP)-1, basic FGF (bFGF), FGFR1, Ang-1, Ang-2, Tie2, HGF, MET, DLL4 and Notch1) in 27 formalin-fixed paraffin embedded (FFPE) human vascular tumour samples evaluated using immunohistochemistry. To identify those growth factors that may be more relevant to malignant tumours, we compared angiogenic growth factor expression in malignant vascular tumours (angiosarcomas) with angiogenic growth factor expression in benign quiescent vascular tumours (haemangiomas), and benign but metabolically active 'inflammatory' vascular lesions (pyogenic granulomas and granulation tissue). We subsequently extended the angiosarcoma tumour expression profile of angiogenic growth factors by studying the expression of 55 angiogenesis-related proteins in a cohort of five fresh angiosarcoma tumour samples using antibody protein arrays.

2. Materials and methods

2.1. Human tumour samples

We studied 39 FFPE vascular tumour samples collected in 2000–2011. This cohort consisted of 18 angiosarcomas (8 breast, 6 cutaneous and 4 soft tissue angiosarcomas), 11 benign haemangiomas and 9 inflammatory vascular lesions (6 pyogenic granulomas, 3 examples of granulation tissue). Tumour samples were identified from the Sheffield Teaching Hospitals pathology archives (REC 09/H1313/30) or collected prospectively (REC 09/H1313/52). Haematoxylin and eosin (H&E) slides from each sample were reviewed to confirm the histological diagnosis, and to ensure the presence of adequate tumour material for immunohistochemistry.

Fresh angiosarcoma tumour samples were collected from five patients following informed consent. Samples were collected perioperatively from patients undergoing surgery for localised disease (REC 09/H1313/52), or from a needle biopsy of advanced lesions prior to axitinib treatment on the phase II Axi-STS clinical trial (ISRCTN 60791336; REC 09/H1208/42). Following biopsy, tumour samples were placed in RNAlater (Qiagen), and stored at −80 °C until analysis.

2.2. Immunohistochemistry studies of archived vascular tumour samples

6 μm tumour sections were mounted on glass slides and dried at 37 °C for 24 h before use. Slides were deparaffinised, and antigen retrieval achieved by heat treating in citric acid (0.01 M citric acid, pH 6.0 with 0.1% Tween) for 10 min before cooling on ice in the citric acid bath for 20 min. Endogenous peroxidase was blocked with 0.3% hydrogen peroxidase (Sigma-Aldrich) in methanol at 37 °C for 30 min. Blocking solution was applied (10% serum, 10% casein) at room temperature for 1 h. The primary antibody was then applied in 2% serum and the slides incubated overnight at 4 °C. The secondary antibody was then applied and the slides incubated for 1 h at room temperature. ABC solution (Elite) was applied for 40 min, and then sections were stained with DAB (Elite) for up to 3 min. Slides were washed in tap water and stained in Gills Haematoxylin for 1.5 min and allowed to 'blue' in running tap water for 3 min. Finally, the slides were dehydrated, coverslips fixed, and sections allowed to dry before scoring. Preliminary studies were performed to optimise the study protocol for each antibody, and the subsequent concentrations of primary and secondary antibodies used are shown in Table 1.

Slides were scored for the number of tumour endothelial cells stained (0 = <1%, 1 = 1–10%; 2 = 11–50%; 3 = >50%) combined with a score for staining intensity (1 = weak, 2 = moderate, 3 = strong). Slides of placental tissue were used as a positive control. Tumour slides processed with the primary antibody substituted with PBS were used as a negative control. Staining of normal tissue adjacent to the vascular tumour was used to assess endothelial specific staining.

2.3. Protein array studies of fresh angiosarcoma tumour samples

Array studies were performed to profile the expression of 55 angiogenesis-related proteins in fresh angiosarcoma tumour samples. Prior to analysis tumour samples were minced in 500 μl of Cell Disruption Buffer (Ambion) using an Ultra-Turrax T8 (IKA Labortechnik) in 15 second bursts. Minced samples were incubated on ice for 2 h, centrifuged at 15,000 rpm for 12 min at 4 °C, and the resultant supernatant collected for array studies. Equivalent protein quantities were analysed using Proteome Profiler Antibody Arrays (ARY007; R&D Systems), performed according to manufacturer's instructions. Duplicate antibody spot peak densities were measured using a GS-710 Calibrated Imaging Densitometer (Bio-Rad) and Quantity One (version 4.6.8) 1-D image analysis software and averaged. Density readings were adjusted relative to an internal positive control and a background reading.

2.4. Statistical analysis

Immunohistochemistry scores were compared between groups using the Mann–Whitney U test, and correlated by calculating Spearman's rank correlation coefficient. Statistical analysis was performed using IBM SPSS Statistics (version 21).

3. Results

3.1. Archived vascular tumour samples

VEGF, VEGFR1, VEGFR2, NRP1, Ang-1, Ang-2, Tie2, bFGF, FGFR1, HGF, MET, DLL4 and Notch1 protein expression were analysed in 39 FFPE vascular tumour samples using immunohistochemistry. VEGFR2, Ang-1, Ang-2 and Tie2 immunohistochemistry staining scores were quantified separately by two independent observers for the complete study cohort. There was good inter-observer agreement between immunohistochemistry scores, which did not differ by more than one grade ($r = 0.846$, 0.826, 0.681 and 0.705 for VEGFR2, Ang-1, Ang-2 and Tie2 respectively, calculated using Kendall's tau-b test).

Immunohistochemistry staining scores of the angiosarcoma samples correlated inversely with the age of the parent tumour block. The

Table 1
Final concentrations of primary and secondary antibodies used for the immunohistochemistry analysis.

Antibody	Primary	Secondary
VEGF; Santa Cruz Biotechnology (sc-152)	1:100	1:200
VEGFR1; Abcam (ab2350)	1:50	1:200
VEGFR2; Abcam (ab39256)	1:100	1:200
NRP1; Abcam (ab81321)	1:250	1:500
bFGF; Abcam (ab106245)	1:250	1:500
FGFR1; Abcam (ab71928)	1:250	1:500
Ang-1; Abcam (ab8451)	1:250	1:500
Ang-2; Abcam (ab65835)	1:75	1:250
Tie2; R&D Systems (AF-313)	1:50	1:250
HGF; R&D Systems (AF-294-NA)	1:250	1:500
MET; Abcam (ab51067)	1:250	1:500
DLL4; Abcam (ab7280)	1:200	1:400
Notch1; Abcam (ab52627)	1:50	1:200

Download English Version:

https://daneshyari.com/en/article/5888361

Download Persian Version:

https://daneshyari.com/article/5888361

Daneshyari.com