Microarray analysis of vascular endothelial growth factor (VEGF)-dependent angiogenic biomarkers in squamous cell carcinoma (SCCA) and adenocarcinoma (AC) of the cervix

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Abstract (#3886)

Objectives: On August 14, 2014, the US Food & Drug Administration approved bevacizumab for advanced cervical cancer based on Gynecologic Oncology Group (GOG) protocol 240, the randomized phase 3 clinical trial which demonstrated significantly improved overall survival with chemotherapy plus bevacizumab compared to chemotherapy alone. Although the signal for efficacy of bevacizumab was not observed in a subgroup analysis of prognostic factors for ACs, this histologic type comprised only 20% of the GOG 240 population. We sought to determine whether VEGF pathway biomarkers were differentially expressed between SCCA and AC of the cervix.
Methods: 244 cervical cancer cases profiled by CARIS were examined retrospectively for changes in gene expression by the Agilent microarray platform. Among the biomarkers studied were VEGFA ligand, VEGF receptors (VEGFR1, VEGFR2), the positive regulator of the VEGF-dependent angiogenic pathway, hypoxia-inducible factor 1 alpha (HIF1α), and the negative regulator von Hippel-Lindau or VHL (involved in ubiquitylation and degradation of HIF1α). The 2-tail Fisher's exact test was performed to test where proportions of positive results were different by subgroup (p≤0.05). JMPv10.0 (SAS Institute Inc., Cary, NC) was utilized for statistical analysis.
Results: The median age for the 158 (65%) cases of SCCA was 47 yrs, and that of the 86 (35%) ACs was 45 yrs. Overexpression of VEGFR1/VEGFR2 was not observed in either cell type (0-2%). Importantly, overexpression of the VEGF ligand was found in 72% and 68% of SCCA and AC, respectively (p=ns). HIF1α was overexpressed in 52% of SCCAs and 41% of ACs (p=ns). VHL was overexpressed in only 26% of SCCAs and 35% of ACs. Co-expression of HIF1α and VEGFA was present in 75% of both SCCA and AC cases.
Conclusions: These data suggest that biomarkers along the VEGF-dependent pathway of tumor angiogenesis are not differentially expressed between SCCA and AC of the cervix. Ligand binding and sequestration using the monoclonal antibody, bevacizumab, is likely to inhibit angiogenesis in women suffering from advanced SCCA or AC of the cervix. The extent to which pre-treatment microarray analysis to guide therapy decisions requires further investigation.

Background

On August 14, 2014, Bevacizumab, the first biological/targeted therapy, was approved for cervical cancer, a disease with limited therapy options after progression on platinum-based regimens. We examined the VEGF pathway for differential expression between SCCA and AC of the cervix.

Methods

Two-hundred forty four cases referred to Caris Life Sciences from 2006 through 2012 were examined retrospectively for changes in gene expression by the Agilent microarray platform. Among the biomarkers studied were VEGFA ligand, VEGF receptors (VEGFR1, VEGFR2), the positive regulator of the VEGF-dependent angiogenic pathway, hypoxia-inducible factor 1 alpha (HIF1α), and the negative regulator von Hippel-Lindau or VHL (involved in ubiquitylation and degradation of HIF1α). The 2-tail Fisher's exact test was performed to test where proportions of positive results were different by subgroup (p≤0.05). JMPv10.0 (SAS Institute Inc., Cary, NC) was utilized for statistical analysis. Ad-hoc analysis of predictive biomarkers by immunohistochemistry (Caris Life Sciences) was performed for additional insight into potential bevacizumab combination strategies.

Results

Figure 1a. Histology (n=244)

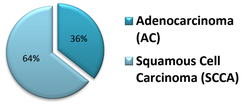


Figure 1b. Disease Status (n=244)

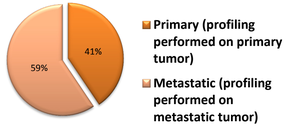
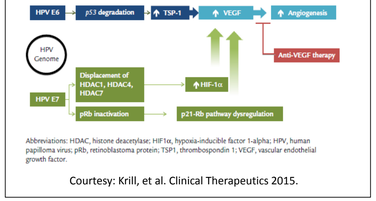


Table 1. Sites of Metastasis	
Specimen Site of Metastatic Cases	% of Metastatic (n=145)
Lymph nodes	23%
Uterus, Nos	12%
Abdomen, NOS	9%
Lung & Bronchus/ Pelvis, NOS, Vagina & Labia	8%
Connective & Soft Tissue	7%
Ovary	6%
Colon/ Liver/Omentum/ Retroperitoneum & Peritoneum	3%
Rectum	2%
Brain/ Chest, NOS/ Kidney/ Small Intestine/ Urinary Bladder/ Vulva, NOS	1%

Figure 2. VEGF-dependent angiogenic pathway



Abbreviations: HDAC, histone deacetylase; HIF1α, hypoxia-inducible factor 1-alpha; HPV, human papilloma virus; pRb, retinoblastoma protein; TSP1, thrombospondin 1; VEGF, vascular endothelial growth factor.

Courtesy: Krill, et al. Clinical Therapeutics 2015.

Figures 1a-b. and Table 1. Patient and Tumor Characteristics. (a) Histological subtypes used for analysis, (b) disease status based on pathology report and specimen provided for profiling. Table 1. Specimen sites used for molecular profiling, representative of metastatic disease status.

Figure 3a. Expression across subtypes (AC+SCCA)

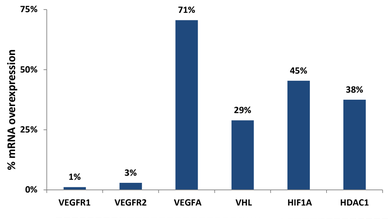


Figure 3b. Histology: AC (green) vs. SCCA (red)

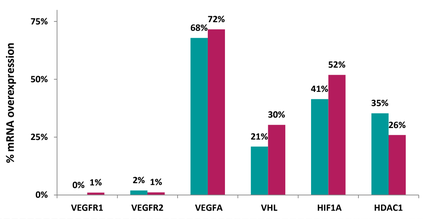
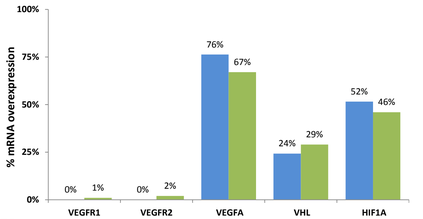


Figure 3c. Disease Status: Primary (blue) vs. Metastatic (green)



Biomarkers along Angiogenesis Pathway

Figure 3a-c. mRNA expression of biomarkers along the angiogenesis pathway. (a) mRNA expression across histology subtypes, (b)mRNA expression patterns in AC vs. SCCA, (c) mRNA expression patterns in primary or metastatic disease states. No statistical differences were found in any comparisons.

Table 2. Genes along Angiogenic Pathway, including oncogenic recetpor tyrosine kinases		
Gene	**Protein**	**Function/Role in Angiogenesis**
ECGF1	thymidine phosphorylase	encodes angiogenic factor which promotes angiogenesis in vivo and stimulates the in vitro growth of a variety of endothelial cells.
EGFR	epidermal growth factor receptor	MAPK pathway, may upregulate VEGF, promoting angiogenesis
ERBB2 (HER2)	human epidermal growth factor receptor 2	MAPK pathway, HER2 signaling upregulates angiogenesis, HER2 overexpression closely associates with increased VEGF expression
PTEN	phosphatase and tensin homolog deleted on chromosome 10	PI3K/PTEN pathway activated by angiogenesis inducers like VEGF, can induce HIF1 and VEGF expression
cMET	hepatocyte growth factor (HGF) receptor	HGF pathway, alternative angiogenesis pathway, cMET overexpression is a marker of angiogenic phenotype
PDGFC	platelet derived growth factor C	PDGF/PDGFRA axis has an important role in angiogenesis, expression correlates with increased vascularity and maturation of vascular wall
PDGFRA	platelet derived growth factor receptor A	
PDGFRB	platelet derived growth factor receptor B	
IGFBP3	insulin-like growth factor binding protien-3	major carrier of IGF, multiple roles in angiogenesis (promoting and suppressing)
IGFBP4	insulin-like growth factor binding protien-4	inhibitor of angiogenesis
IGFBP5	insulin-like growth factor binding protien-5	inhibitor of angiogenesis
SRC	Src-family kinases	deregulation of expression may lead to pro-angiogenic molecules, Src-family kinases may be required during VEGF-induced angiogenesis
YES		
FYN		
TNF	tumor necrosis factor alpha	stimulates vessel growth
PTGS2(COX2)	cyclooxygenase-2	important mediator of angiogenesis, pro angiogenic effects mediated primarily by arachidonic metabolism

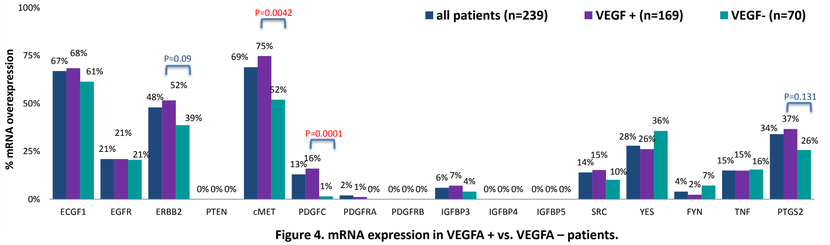


Figure 4. mRNA expression in VEGFA + vs. VEGFA – patients.

Table 2. and Figure 4. Oncogenes and Angiogenesis. VEGF expression is upregulated through activation of tyrosine kinase receptors including EGFR, HER2, PDGFRA and through activation of the MAPK and PI3K/PTEN pathways. mRNA expression patterns of oncogenes and biomarkers involved in angiogenesis were assessed for differences between VEGFA+ and VEGFA- cervical cancer patients. VEGF+ status was statistically correlated with overexpression of cMET (75% vs. 52%; p=0.0042) and PDGFC (16% vs. 1%; p=0.001). ERBB2 and PTSGS2 (COX2) overexpression correlation with VEGFA+ status was trending towards statistical significance as well.

Results

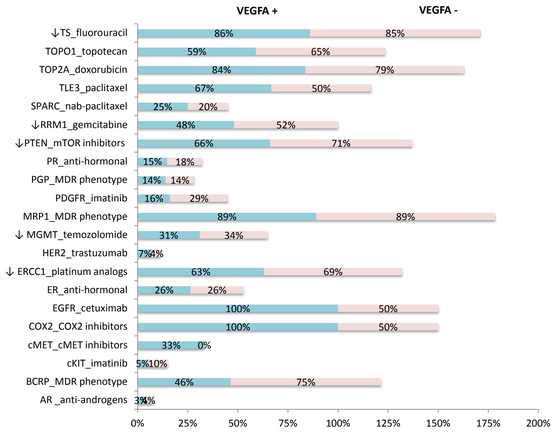


Figure 5. Expression of predictive biomarkers by Immunohistochemistry

Conclusions

- These data suggest that biomarkers along the VEGF-dependent pathway of tumor angiogenesis are not differentially expressed between SCCA and AC of the cervix.
- Overexpression of the ligand, VEGFA, and not the receptor, VEGFR1/2 modulates angiogenesis in cervical cancer
- Ligand binding and sequestration using the monoclonal antibody, bevacizumab, is likely to inhibit angiogenesis in women suffering from advanced SCCA or AC of the cervix.
- The extent to which pre-treatment microarray analysis to guide therapy decisions requires further investigation.

References

1. Kirll, L.S., K.S. Tewari. (2015). "Exploring the Therapeutic Rationale for Angiogenesis Blockade in Cervical Cancer." Clinical Therapeutics 37(1): 9-19

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