Evidence Based Hypothesis: ALPHA

(Acidic Lactate sequentially induces Lymphogenesis, Phlebogenesis, Arteriogenesis)

a new Vasculogenesis concept for Glycolysis# which Better explains imaging perfusion measurements

#Haaga JR, Haaga RE, ALPHA Glycolytic Vasculogenesis, Surgery, September, 2013

- John Haaga MD, FACR, etc., Tenured Professor, Chairman Emeritus

Case Western Reserve University
Thousands of vasculogenesis reports like trees in a forest. Confused/lost because there are so many! ALPHA is a new “map”
DISCUSSION

- 5000 references in high impact journals provide evidence basis for theory
- Brief Review Traditional and ALPHA vasculogenesis
- Inconsistencies of Traditional Theory
- Biochemical and molecular basis of ALPHA
- Modern imaging perfusion more consistent with ALPHA
- Preliminary proof of ALPHA
- “Real” biomarkers correlate with ALPHA
Traditional Theory: 1) Cancer uses aerobic requiring oxygen. 2) Becomes hypoxic when grows larger than 1-2 mm 3) Hypoxia slows growth & causes dormancy. 4) Hypoxia induces HIF which induces VEGF 5) VEGF stimulates arteries to restore normoxia and tumor growth

ALPHA Theory: 1) Cancer uses aerobic AND glycolysis provides many pro-cancer advantages. 2) Excessive lactate impairs glycolysis which precludes advantages. 3) Lymph/venous drainage needed to manage lactate 4) Lactate increases HIF by 3 mechanisms and VEGF, FGF by other paths 5) Vessels develop sequentially as first lymphatics, next veins, then arteries
Inconsistencies of Traditional Theory

- With growth or loss of arterial flow cancers become glycolytic which helps cancers. After tumors grow $>2\text{mm}$ they become hypoxic/glycolytic and more aggressive.

- Rx with anti-VEGF drugs or embolization improve tumors temporarily but usually recur in more aggressive form.

- Imaging perfusion measurements are not consistent with arterial theory. Arterial peaks and flow not useful, more consistent with ALPHA, i.e. lymph, veins.

- When tumors outgrow arterial supply, O2 drops but tumors continue growth by glycolysis
Over Time, changes occur flow drops

R-correlation between tumor growth and arterial perfusion is negative. Glycolysis supports further growth.
Scans confirm glycolytic tumors grow.

Literature confirms hypoxia and glycolysis help cancer growth/invasion/metastases.

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EDITORIALS

Tumor Hypoxia: Chicken, Egg, or a Piece of the Farm?

C. Norman Coleman, James B. Mitchell, Kevin Camphausen
After occlusion of veins and arteries, tumor becomes hypoxic and glycolytic

Cell type changed from pleomorphic cells to spindle shape with fewer mitochondria, and cellular motility with invasive traits.

Increased local invasion- cell motility (lactate > hyaluronan > motility - Maxwell)

Increased alternate angiogenic factors
Mechanism: lactate modulates invasion

Lactate > CD44 > Hyaluronan > RHAMM > causes motility and invasion of cells

A: aerobic tumor with no invasion

B: glycolytic tumor with motility and invasion, arrow

Keunen O et al. PNAS 2011;108:3749-3754
Tumor Arterial Embolization (particles, chemo, or radioactive Ytrium) temporarily reduces cancers but recur with aggressive glycolytic phenotype.
Aerobic/Glycolysis need different vessels

- Aerobic metabolism using 1 glucose and 1 O2 efficiently makes 38 ATP’s and 2 CO2. **ARTERIES NEEDED**
- Glycolysis using 1 glucose and **NO** oxygen makes 2 ATP’s and 2 Lactate. For 38 ATP also 38 Lactate. **100X FASTER** because feed forward & fewer reactions

NEED LYMPH & VEINS to manage lactate:

Moderate lactate levels support many pro-cancer advantages.

High lactate slows glycolysis by feedback impairing cancer advantages
Glycolysis/moderate lactate aids cancer

ATP production 100x faster than aerobic, few reactions & feed forward mechanism


Mother cell proliferation depends on growth substrates (proteins, DNA, etc) which come from side reactions of glycolysis

Moderate level of Lactate is a powerful pro cancer modulator: increases HIF, motility, invasion, mutation, immune escape, anti-apoptotic,

HIGH LACTATE STOPS GLYCOLYSIS IMPAIRING CANCER
High Lactate stops glycolysis by end product inhibition and low pH inhibition of phosphofructose Kinase

- High lactate decreases ATP and substrate supply (lipid, protein, nucleotide supply) so no growth

- If mother cells can’t grow to double biomass for daughter cells they do not divide/proliferate but lapse into G0 (Van Heiden “Science” 09, Weinberg 09).

- TO AVOID EXCESS LACTATE GLYCOLYTIC TISSUE MUST DEVELOP LYMPHATICS AND VEINS; LACTATE VASCULOGENESIS
Science is too specialized, isolated in information “stovepipes”. Like blind men feeling elephant. ALPHA integrates data explaining glycolytic vasculogenesis.
Lactate stimulates vasculogeneisis by vascular growth factors, HIF, ROS, and attraction of stem angioblasts.

Sequence of vessel development is lymphatics first, veins then arteries.

JRH animal experiment (adapted from animal model of Indraccola et al " Interruption of tumor dormancy by angiogenic burst from microenvironment.", PNAS, 2006)
Example reports of how Lactate/pH induces Vascular Growth Factors


Hunt et al,... “Aerobically derived lactate induces VEGF, TGFb (transforming growth factor), IL-1, and HIF, Antioxid. Redox.Signal, **2007**;9(8),p102

Vegran F, Boidot R, Michiels C, “Lactate Influx ... drives angiogeneis.” Increase FGFx2, IL8x8 fold, NFkB

*Cancer Research* **2011**;71,p2550-
Sequence of Vessel Development: lymphatics first, veins then arteries

1) Transfected VEGF gene-Lab. Invest (Nature Group) Impact 3.6
2) Growth factor pellet implant in Cornea Proc Nat Acad Science Impact 9.7
3) Denovo skin cancer model-Cancer Research, Impact 7.8
4) Xenograft implant in mouse-Circulation Research, Impact 9.5
FGF2 induces VEGFC at lowest levels and VEGFA at higher levels, **Impact 9.6**


Chang et al, *PNAS*, 2004, 101(32) senior author Kaipainen, Med Nobel Institute, Stockholm,
First Blood vessels formed are veins “cannot have flow in without flow out”

Why should a radiologist care?

Because vascular perfusion parameters from MRI, CT, PET, and ultrasound are more consistent with ALPHA:

1) Diffusion permeability - $K_{\text{trans}}$, $K_{\text{ep}}$, Patlack - Depends on Veins

2) Contrast Washout and kinetic curves depend on increased veins

3) Blood volume - predominantly veins
Simultaneous measurement of oxygenation and Ktrans permeability, Matsumoto et al, PNAS, 2009 vol. 106 no. 42, 17898-17903. NO correlation between oxygen and permeability
Permeability occurs thru veins - Ktrans

“All tracers leaked primarily from venules and small veins at the tumor-host interface, for the most part vessels lined by a continuous endothelium. The predominant pathway by which all four tracers exited venules in all three tumors”  
Kohn et al  Lab Invest. 1992 Nov;67(5):596
Kinetic curves for MRI DCE breast depend on venous outflow, Kuhl et al, Radiology, 1999;211:101-110
Blood volume and permeability values on MRI or CT useful for differentiating tumors

- Duong et al calculated components of blood volume to be 29% arterial and 71% venous. Hence, mostly reflects venous system.
Spaminato et al (Radiology) reported degree of differentiation predicted by Blood Volume

Low blood volume map indicates low blood volume low grade oligoastro

Relative cerebral blood volume map shows elevated vascularization-anaplastic oligodend
Blood volume is increased, via vein: vascular biomarkers


Noren et al, PNAS, 2004

*Ephrin B4* increases vessel size, blood volume and tumor growth. (CONSISTENT with ALPHA)

Overexpression *B2* suppresses tumor growth (CONTRARY TO OLD THEORY)

Same results in 10 additional reports
Preliminary Proof of ALPHA

- We duplicated experiment by Indraccola et al (PNAS), which converted non angiogenic tumor MOLT3 to growing vascular tumor.
- MOLT3 alone didn’t grow, MOLT3 with VEGF or FGF grew, and MOLT3 with irradiated sarcoma cells grew.
- They concluded dormancy interrupted by angiogenic burst from microenvironment.
- Haaga et al, studied the same “dormant”, non-growing, non-angiogenic cell line MOLT3 using lactate.WHICH GREW
“Interruption of tumor dormancy (MOLT3) by a transient angiogenic burst within the microenvironment” Indracolla et al PNAS, 2006
Haaga Experiment
Repeated MOLT3 model
Using same cell line cohorts in SCID mice except included group with MOLT3 and Lactate.

Molt3 cells
Lactate (30mg/ml)

Molt3 cells only
No Tumor
Xenograft tumors grown in NOD SCID Mice

Molt3 cells (5x10^6) + KS cells (5x10^6, irradiated 45 Gy before s.c. injection into the flanks of NOD SCID mice. P = 0.0546 (between groups of molt3+KS) and molt3+lactate

Molt3 cells (5x10^6) + extracellular matrix gel + Lactate (30 mg/ml). P< 0.05 (between groups of molt3+lactate and molt3 only

Molt3 cells (5x10^6) + extracellular matrix gel
Future Applications

- New Imaging methods related to DWI and ADC. Relates to lactate production and interstitial edema.
- Improved treatment: instead of treating only the aerobic metabolism should treat both aerobic and glycolytic.
Blocking waste enzymes changes glycolysis, lactate, interstitial fluid & ADC

Liposarcoma pre and post drug-116 to 72

Metastatic breast to kidney-215 to 122
Rx of liver cancer is most effective if both aerobic and glycolytic treated simultaneously.

Fig a Control group and Rx group. Fig b. Treatment groups: TAE-AG-B and TAE-C best.
Folkman was a friend to many BUT
Original report has ??? Statistics

National Cancer Institute
National Institutes of Health
6130 Executive Boulevard, MSC 7412
Bethesda, MD 20892

Ladies/Gentlemen:

This is in regard to the grant entitled, “Use of vasoactive agents to enhance specificity of CT/MRI imaging”; application number 1 R21 CA1155801A1. I have reviewed the preliminary data, submitted for the grant by Dr. Haaga and his team. I find this principle and concept very exciting and it has great possibilities for the future.

It is my belief that Dr. Haaga’s research will impact the diagnosis of tumor chemotherapy assessment and treatment, and even translation to the area of biomarkers.

If this grant is approved, I would be happy to be a consultant to Dr. Haaga as he pursues the development and refinement of this principle.

Sincerely yours,

Judah Folkman, MD
Graph of single animal used for conclusions

Arrow shows onset of arterial flow via injection of fluorescein

Growth precedes flow in this single animal. Other data from 10 animals re-evaluated by Tien and Chankung

Fig. 5. The characteristic growth curve of an iris implant (BP No. 29R) plotted on a semi-logarithmic scale. Positive fluorescein test on day 6 represents earliest evidence of perfusion of the tumor, and coincides with the beginning of exponential volume increase. Slopes “a,” “b,” and “c,” corresponding to prevascular, vascular, and late phases of growth, are indicated.
10 animals studied, but their conclusions drawn from one animal/graph not 10.

### TABLE I

**Rates of Growth and Time of Vascularization of 10 Iris Tumors**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Growth curve slopes*</th>
<th>Primary inflection (a to b)</th>
<th>Vascularization‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Prevascular) (a)</td>
<td>(Vascular) (b)</td>
<td>(Late) (c)</td>
</tr>
<tr>
<td>1</td>
<td>0.04</td>
<td>0.49</td>
<td>0.23</td>
</tr>
<tr>
<td>2</td>
<td>0.05</td>
<td>0.68</td>
<td>0.14</td>
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<tr>
<td>3</td>
<td>0.08</td>
<td>0.58</td>
<td>—</td>
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<tr>
<td>4</td>
<td>0.03</td>
<td>0.96</td>
<td>0.14</td>
</tr>
<tr>
<td>5</td>
<td>0.04</td>
<td>0.87</td>
<td>0.25</td>
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<tr>
<td>6</td>
<td>0.16</td>
<td>0.54</td>
<td>0.13</td>
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<tr>
<td>7</td>
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<tr>
<td>9</td>
<td>0.06</td>
<td>0.64</td>
<td>0.12</td>
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<tr>
<td>10</td>
<td>0.12</td>
<td>0.56</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Mean ± sd = 0.085 ± 0.055  0.65 ± 0.155  0.16 ± 0.051

* Estimated from semilogarithmic plots (see text).

‡ “Positive” fluorescein test or gross appearance of tumor vessels.
Using a statistical t-test at 95% confidence level, it confirms that arterial vascularization occurs approximately 1 day after the exponential growth begins. In conclusion, based on the data in Grimbrone et al. (1972), the tumors appear to BEGIN its rapid exponential growth about 1 day prior to arterial vascularization. If this is true, then there must be a mechanism other than arterial vascularization that serves as a primary factor to stimulate the abrupt growth.
Research supported by John and Ellen Haaga thru Elizabeth Haaga Memorial Fund CWRU

?? Know any foundations which fund “out of the box” concepts….NIH doesn’t
Improved RX of Hepatocellular Carcinoma in Rat with bimodal Rx of aerobic and glycolytic metabolism.
Collating many “overlooked” data from high impact journals support ALPHA. “Lactate” is the 800 Lb Gorilla “overlooked” in the angiogenesis room.

For PDF, please email John.Haaga@uhhospitals.org

Please view computer lecture which has more details. Original report by Gimbrone and Folkman has serious statistical issues.

- Small tumor implants inserted into two sites, anterior chamber and ciliary body with vessels
- Tumor size monitored with ophthalmic slit lamp
- Onset of Arterial flow detected by intravenous fluorescein.
Conclusion

Cancer prefers and requires glycolysis because it and lactate provide pro cancer beneficial processes.

ALPHA Vasculogenesis complements the traditional vasculogenesis concept because it explains the role and mechanism for glycolytic vasculogenesis.

To avoid cellular arrest caused by excess lactate, vascular growth factors develop lymphatics first, veins, and then arteries.
Kaposi and MOLT3Lac  MVD by CD31 same

MOLT3+KS d40 (A)  MOLT3+KS d40 (B)  MOLT3+Lac d40 (A)

MOLT3+Lac d40 (B)  MOLT3+Lac d40 (C)  MOLT3+Lac d40 (D)
ALPHA – Ki-67 + Cleaved Casp3 IHC Results, differs from Indraccola et al, same vessels but remarkable proliferation and apoptosis.
THE END
“Using a statistical t-test at 95% confidence level, it confirms that arterial vascularization occurs approximately 1 day after the exponential growth begins. In conclusion, based on the data in Grimbrone et al. (1972), the tumors appear to BEGIN its rapid exponential growth about 1 day prior to arterial vascularization. If this is true, then there must be a mechanism other than arterial vascularization that serves as a primary factor to stimulate the abrupt growth.”
Too much data, alphabet jargon:
Little integration of data
“Traditional Mantra” is tumors become hypoxic when they grow larger than 2mm

Cancer uses glycolysis in normoxia or hypoxia, because of pro cancer advantages
LACTATE INDUCES HYALURONAN
Is Traditional Vasculogenesis the “law” or other principles needed?

Many inconsistencies of the traditional theory exist in both RX and imaging.
Scandinavian Contributions

- Vascular endothelial growth factor C induces angiogenesis in vivo Cao, Yihai, Alitalo, K, PNAS November 24, 1998 vol. 95 no. 24 14389-14394
