Cancer as a disease of development; Developmental therapies: Anti-Angiogenesis; Stem cells and tissue regeneration

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What is Cancer?

- Unrestricted cell growth: tumor cell population $1 \times 10^9$ cells
- Mutations cause enhanced cyclins or inhibited p16 leading to unrestricted cell cycle
- Mutation in p53 inhibits apoptosis
- Metastasis
• Cancer cells have abnormal cell cycles
  – divide excessively and form tumors
The cancer stem cell model

- Cancer stem cell
- Transient amplifying cells
- Differentiated cells

The stochastic model

- Self renewal
Tumour

- Unspecific therapy
- Tumour shrinkage
- Relapse

Therapy targeting cancer stem cells

- Tumour shrinkage
- Tumour regression
Angiogenesis: Cascade of Events

1. Angiogenic factor production
2. ...release...
3. EC receptor binding
   - Intracellular signalling
4. EC activation
   - BM degradation
5. EC proliferation
6. Directional migration
7. ECM remodeling
8. Tube formation
9. Loop formation
   - a = differentiation
10. Vascular stabilization

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Process of Angiogenesis

• Induction
  – Vasodilation and increased permeability of preexisting vessels
  – Activated endothelial cells release proteases to degrade matrix
  – Endothelial cells proliferate and migrate
  – Proliferating cells adhere to one another

• Resolution
  – Differentiation and maturation of blood vessels
History of Antiangiogenic Drugs

• 1971: The field began in early 1970s with Judah Folkman’s hypothesis that tumor growth would be halted if it were deprived of a blood supply
• 1989: Dr. Napolene Ferra identified and isolate VEGF
• 1996: Dr. Jeffery Isner published first clinical trials regarding VEGF
• 2004: FDA approves first antiangiogenic drug to treat colorectal cancer (Avastin)
Antiangiogenesis Targets

• Neovasculature
  – 1. Proteases that breakdown the ECM (e.g., MMPs)
  – 2. Growth factors that stimulate endothelial cell proliferation (e.g., VEGF)
  – 3. Integrins that allow adhesion of endothelial cells
  – 4. Endothelial cell apoptosis

• Preexisting Vasculature
  – 5. Various Vasculature Targeting Agents
Neovasculature: Inhibiting ECM Breakdown

• MMPs (metalloproteinases) are proteolytic enzymes that cleave the basement membrane
• Three domains: pro-peptide, catalytic domain, haemopexin-like c-terminal domain
MMP-Inhibiting Drugs

• Marimastat (left)
  – Binds to zinc ion
  – Very limited success due to toxicity factors and need for cytotoxic combination

• Batimastat (right)
  – 1,4 bidentate hydroxamic acid ligand that binds very tightly to the zinc ion in the catalytic (active) site
Neovasculature: Inhibiting cell growth

- Tumor cells are hypoxic, which induces HIF1 to signal over production of growth factors
- Target the growth factor
  - VEGF, PDGF, bFGF, IL-8
- Target the growth factor receptor
Drugs Preventing Cell Proliferation

- Suramin--prevents bFGF and VEGF from binding to the active site of their receptors through competitive inhibition

- Avastin--antibody that targets VEGF (binds to VEGF to inhibit VEGFR1 and VEGFR2)
  - Enables normalization: reduced blood vessel permeability and interstitial pressure

- Angiostatin--binds to HGF (hepatocyte growth factor); blocks endothelial cell surface ATP-synthase
VEGF Is a Key Mediator of Angiogenesis

Upstream activators of VEGF synthesis

Downstream signaling pathways

VEGF

Endothelial cell

Survival

Migration

Proliferation

ANGIOGENESIS
Methods of VEGF Signal Inhibition

Ligand sequestration: MAbs, soluble receptors

Receptor blocking MAbs, soluble receptors

Inhibit receptor production; ribozymes

Tyrosine kinase inhibition: TKIs

Transcription factor inhibition

Indirect - inhibition of growth factors, HER-2

Inhibition of tyrosine phosphorylation and downstream signaling inhibition

PlCγ

p85

GRB2

SOS

ras
rhuMAb VEGF (Recombinant Humanized Monoclonal Antibody to VEGF)

- Humanized to avoid immunogenicity (93% human, 7% murine).
- Recognizes all isoforms of vascular endothelial growth factor, \( K_d = 8 \times 10^{-10}M \)
- Terminal half life 17-21 days
Toxicities of Anti-VEGF Therapy

• Hemorrhage (lung cancers)
• Hypertension (anti-VPF)
• Headaches/migraines
• Clots (maybe)
• Proteinuria/nephrotic syndrome
Mechanisms of Resistance

• Endothelial cell heterogeneity
• Tumor heterogeneity
• Impact of tumor microenvironment
• Compensatory response to hypoxic insults
• Re-growth independence from angiogenesis
• Vascular mimicry
• Vasculogenesis
Thwarting Resistance

- Use chemotherapy with anti-angiogenic intent - ‘metronomic therapy’
- Combine with chemotherapy
- Combine multiple anti-angiogenics
- Combine with other biologics
- Use anti-angiogenics as targeted therapy
- Use anti-angiogenics in adjuvant setting
Neovasculature: Inhibiting Cell Adhesion

- Integrin avb3
  - Arginine-glycine-aspartic acid containing ligand binds and causes conformational changes
  - Targets:
    - Antibodies against avb3 ligands
    - Integrin binding antagonists
    - siRNA
Integrin Antagonists

- **Cilengitide**
  - Avb3 antagonist
  - Contains the RGD sequence and blocks the ligand

- **LM-609; Vitaxin 2**
  - Avb3 antibodies
Neovasculature: Inducing apoptosis

• Target: Tumor Necrosis Factor--causes endothelial cell apoptosis in tumor cells (induces inflammation and endothelial cell growth in normal cells)

• Target: Down-regulating/blocking Bcl-2 interactions with pro-apoptotic proteins
  – Endostatin
  – Angiostatin
Neovasculature: Other Novel Agents

- Celecoxib: COX-2 (cyclooxygenase-2) Inhibitor
  - Common use: arthritis treatment (Celebrex)
  - decrease vascular permeability
  - decrease EC proliferation
  - decrease EC migration
  - decrease MMP production
  - affect integrin pathway
• Thalidomide
  – Discontinued use: treat morning sickness
  – FDA approved in 2006 for combination therapy with dexamethasone for treatment of multiple myeloma (cancer of plasma cells)
    • Block bFGF and VEGF
    • Inhibit COX-2
    • Interferes with Tumor Necrosis Factor-alpha
Preexisting Vasculature: VTAs

- Vasculature Targeting Agents disrupt already-present blood vessels
- New field of antiangiogenesis research
- **Combretastatin A-4** (prodrugs: CA4P and Oxigene) destabilizes microtubules of vascular cells
- **DMXAA** (Flavonoid analog) increases NF-kb transcription by phosphorylation leading to the production of proteins that change vascular cell shape and organization eventually leading to apoptosis of these cells
Potential for Antiangiogenesis

• COMBINATION THERAPY
  – Antiangiogenic+chemotherapeutic drug
  – Inhibit vascularization+cytotoxic agent
  – Avastin+PDGFR inhibitor
    • Avastin clinical dose=5-10mg/kg
      – Dose limiting toxicity=20mg/kg
    • Selection against Avastin
  – Thalidomide combinational therapy
Stem cells and tissue regeneration

• Regenerative medicine is the promised paradigm of replacement and repair of damaged or senescent tissues.

• Based on the promise of being able to engineer cells and tissues of the human body to restore parts lost to trauma or disease.

• Stem cells have unique and wide-ranging capabilities
Regeneration in Nature

• Outstanding Examples
  – Planarian
  – Crayfish
  – Embryos

• Inverse Relationship
  – Increase complexity
  – Decrease regenerative ability
Regeneration in Humans

High

Moderate

Low
How Regeneration Works

• Adult stem cells normally remain quiescent (non-dividing) for relatively long periods of time until they are activated by signals to maintain tissues.
• When activated they divide through a process called asymmetric cell division.
• Through this process they are able to maintain their populations and differentiate into the desired cell types by the creation of a progenitor cell.
• A progenitor cell, in contrast to stem cells, is already far more specific: they are pushed to differentiate into their "target" cell.
Regenerative Medicine

• Process of creating living, functional tissues to repair or replace tissue or organ function lost due to age, disease, damage, or congenital defects

• It helps to produce extended healthy longevity,
Clinical Needs

• Cardiovascular
  – Myocardial infarction
  – Stroke

• Bone
  – Non-union fractures
  – Tumor resections

• Nervous
  – Spinal Cord Injury
  – Degenerative diseases
Stem Cells

- Long-term self-renewal
- Clonogenic
- Environment-dependent differentiation
Tissue Engineering

- Repair/replace damaged tissue
  - Enhance natural regeneration

**Cell Source**
- Embryonic stem cells
- Adult stem cells
- Progenitor cells

**Signals**
- Growth factors
- Drugs
- Mechanical forces

**ECM**
- Metals
- Ceramics
- Synthetic polymers
- Natural polymers
Important Variables

• Delivery
  – Cell Suspensions
  – Tissue-like constructs (scaffolds)

• Chemical properties
  – Growth factors
  – Degradation particles
  – ECM surface

• Physical properties
  – Structure
  – Topography
  – Rigidity
  – Mechanical Loading

Modify Cell Behavior
  Survival
  Organization
  Migration
  Proliferation
  Differentiation

Optimize Cellular Response
Stem and Progenitor Cells

• Isolation/Identification
  – Signature of cell surface markers
  – Surface adherence
  – Transcription factors

• Classifications
  – Embryonic Stem Cells
  – Adult Stem Cells
  – Induced Pluripotent Stem Cells
Embryonic Stem Cells

**Strengths**
- Highest level of pluripotency
  - All somatic cell types
- Unlimited self-renewal
  - Enhanced telomerase activity
- Markers
  - Oct-4, Nanog, SSEA-3/4

**Limitations**
- Teratoma Formation
- Animal pathogens
- Immune Response
- Ethics
Potential Solutions

• Teratoma Formation
  – Pre-differentiate cells in culture then insert

• Animal pathogens
  – Feeder-free culture conditions (Matrigel)

• Immune Response
  – Somatic cell nuclear transfer
  – Universalize DNA

• Ethics
Adult Stem Cell

- Undifferentiated Cells
- Found throughout the body after embryonic development
- Multiply by cell division to replenish dying cells
- Regenerate Damaged Tissues.
Types of Adult Stem Cells

- Hematopoietic
- Mammary
- Mesenchymal
- Neural
- Endothelial
- Olfactory
- Neural crest
- Testicular
Properties

• Defining properties - self-renewal & potency
• Lineage
• Signaling pathways
• Multidrug resistance
• Plasticity / Transdifferentiation
Adult Stem Cells

**Strengths**
- Ethics, not controversial
- Immune-privileged
  - Allogenic, xenogenic transplantation
- Many sources
  - Most somatic tissues

**Limitations**
- Differentiation Capacity?
- Self-renewal?
- Rarity among somatic cells
Potential Solutions

- Differentiation Capacity
  - Mimic stem cell niche
- Limited Self-renewal
  - Gene therapy
- Limited availability
  - Fluorescence-activated cell sorting
  - Adherence
    - Heterogenous population works better clinically
Mesenchymal Stem Cells

- Easy isolation, high expansion, reproducible
Hematopoietic Stem Cells

- Best-studied, used clinically for 30+ years
Induced Pluripotent Stem Cells

**Strengths**
- Patient DNA match
- Similar to embryonic stem cells?

**Limitations**
- Same genetic pre-dispositions
- Viral gene delivery mechanism
Potential Solutions

• Same genetic pre-dispositions
  – Gene therapy in culture

• Viral gene delivery mechanism
  – Polymer, liposome, controlled-release

• Use of known onco-genes
  – Try other combinations