Collaboration in Clinical Research: A Critical Ingredient

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Clinical Research

- The definition of Clinical Research is very broad
- Encompasses – clinical trials, outcomes, health delivery, epidemiological and psychosocial research
- Translational clinical research focuses on bench-to-bedside interface and requires investigators schooled in necessary skills

Translational Clinical Investigators

- **At least two flavors:**

  - **Patient Oriented Research (POR)**
    - Actively interact with patients who may enable them to uncover secrets of complex diseases
    - Care for those patients
    - Develop new therapeutic or diagnostic approaches

  - **Disease Oriented Researchers (DOR)**
    - Interested in disease mechanisms
    - Do not interact patients/subjects in their research
    - Study tissue samples, cell lines, model systems such as mice, fish, yeast
Several Cs of Translational Clinical Research

David G. Nathan, M.D.

*J Clin Invest* 115:795-797, 2005

- Clinical Focus
- Collaboration
- Courage – to learn new techniques/approaches
- Critical Awareness of literature
- Constructive Infrastructure
- Consent from Patients
- Conflict of Interest
- Caring Mentors
One Route to Successful Patient Oriented Research: Collaboration

• “It is increasingly difficult for a single individual simultaneously to fill the roles of physician and scientist. One sure way to cover the spectrum: Collaboration.”

• “We are referring to an intimate collaboration between two individuals that allows them jointly to cover a range that neither could cover alone”

Joseph L. Goldstein, M.D. and Michael S. Brown, M.D.

Nobel Laureates
Research Interests: Rao Lab

- Molecular mechanisms of inherited platelet function.
- Alterations in blood coagulation mechanisms in health and disease.
- Impact of antithrombotic agents on platelets and coagulation systems.

Basic and Clinical Research
Molecular Basis of Inherited Platelet Function Disorders
AKR 2011

Secretion

Phospholipids

Arachidonic Acid

PGG2/PGH2

TxA2

CO

PLA2

TS

ADP (P2Y1)

Gq

ADP (P2Y12)

ATP

Gq

Ple

AC

cAMP

PKC

ADP (P2Y12)

Gq

ADP (P2Y1)

Gq

DG

Thrombin

PLC

Gq

PIP2

IP3

Thromboxane

PAF

Gq

Gq

Gq

Ca

Collagen

TK

Ca

MLC

Ca

PKC-θ

Sun, L. et al, Blood 103: 948-954, 2004

PHOSPHOLIPASE C--β2 (PLC-β2)


Mao, GF et al, Blood 99: 905-911, 2002

Gαq


GPIIb-IIIa

AKR 2011
The Long Story of studies in one patient

...In Short

3 years old white male

Thrombocytopenia

Platelet Function Defect

Father had died of Acute Leukemia
Decreased activation of GPIIb-IIIa

Decreased phosphorylation of Pleckstrin and Myosin Light Chain

ADP (P2Y12) → cAMP

ADP (P2Y1) → DG

Thrombin → PLC

Thromboxane → DG

PAF → DG

Collagen → Ca

Ca → IP3

IP3 → PLC

PAF → Phospholipase A2 (PLA2)

PGG2/PGH2 → PGD2

Arachidonic Acid

Collagen

Adenosine diphosphate (ADP) (P2Y12)

Gq

AC

Gq

Pleckstrin

PKC

ATP

Plasma membrane

AGGREGATION

GPIIb-IIIa

ADP (P2Y12)

Fibrinogen

Reduced phosphorylation of Pleckstrin and Myosin Light Chain

Decreased activation of GPIIb-IIIa

Protein Sequence:

WT: KKVVALGDVPDGLTV.......AFNPQPSQMQDT..

Patient: GMFQMALWSL*

RUNX1 Immunoblot:

P N N N N

Sun et al, *BLOOD*, 103: 948-954, 2004
What are Transcription Factors??

DNA → mRNA → Protein

- Each cell contains a complete copy of organism’s genome
- What makes cells different?
  - Each cell utilizes and expresses only a subset of genes
  - Differential expression is regulated at transcriptional level
Questions

• What are the genes regulated by transcription factor RUNX1 in platelets/megakaryocytes?

• This would help us understand the mechanisms leading to the platelet dysfunction and the thrombocytopenia.
ACT 3
Approach: Genome-Wide
Platelet Expression Profiling
Platelet Expression Profiling

- Expression profiling permits assessment of gene expression (mRNA)
- Established a collaboration: Children’s National Medical Center, Washington, DC
- Affymetrix U133 human genomic chip sets (A and B). ~44,000 probe sets.
- Platelet total RNA isolated ~350 ml blood

Platelet Expression Profiling: Findings

- 70 genes down-regulated
- Most down-regulated gene was MYL9 (myosin light chain)
- Revealed alterations in expression of genes we could not have predicted
- Genes whose function not even known in platelets

Are these regulated by RUNX1 at Transcriptional level?

Myosin Light Chain (MYL9)
Jalagadugula et al *Blood* 2010;116:6037-6045

12-Lipoxygenase (ALOX12)
Kaur et al *Blood* 2010;115:3128-3135

Platelet factor-4 (PF4)

Protein kinase C-θ (PKC-θ)
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HYPERGLYCEMIA AND HYPERINSULINEMIA

INDUCED PROCOAGULANT STATE IN

DIABETES MELLITUS
Patients with DM have increased atherosclerotic and acute vascular events.

The rupture of the atherosclerotic plaque is a major cause of sudden death in humans.

Diabetes mellitus is a procoagulant state.

Both high glucose and high insulin levels are independently associated with increased mortality.

The effects of hyperglycemia and hyperinsulinemia on blood coagulation are ill-defined.
STUDY DESIGN

- **Hyperglycemia and Hyperinsulinemia (HG+HI):**
  - 10 healthy individuals
  - Infused with glucose (~200 mg/dl) for 24 hrs;
  - Endogenous insulin ~ 1000 pM.

- **Euglycemia and Hyperinsulinemia (EG+HI):**
  - 7 individuals
  - Infused with regular insulin (~1000 pM), and glucose (100 mg/dl) for 24 hrs.

- **Selective Hyperglycemia and Euinsulinemia (HG+EI):**
  - 6 individuals
  - Infused with high glucose (200 mg/dl); and Somatostatin to inhibit endogenous insulin release.

- **Euglycemia and Euinsulinemia (EG+EI) Placebo:**
  - 5 individuals: infused with saline.
Tissue Factor Procoagulant Activity In Whole Blood

High Glucose/High Insulin

Platelet

↑ CD40L

EC-Inflammation

↑ TF

Monocyte-TF

↑ mRNA/protein

CD40L

TFPCA

Hypercoagulable State

↑ TAT, ↑ F1.2, ↑ FVIII, ↓ FVIIa

Thrombin

ATHEROSCLEROSIS

Vaidyula VR et al.

Diabetes 55:202-208, 2006
Translational Clinical Research: Power of Collaboration

• Collaborations are essential

• Essential for moving forward in the world of exploding information and technology

• “It is crucial that they interact as equals, each contributing ideas...Partnerships based on subservience are doomed to failure” (Goldstein, J and Brown, M, J Clin Invest 1997, 99: 2803-2812)

• Not all collaborations work out
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The future of medicine absolutely depends on clinical investigators
Thank you