Identification of a Novel Biomarkers for the Diagnosis and Treatment of Hemorrhagic Shock

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Basic Concepts

- Diverse response to hemorrhage/trauma
- Innate pro-survival pathways
- Creating a “pro-survival” phenotype
- Pharmacological resuscitation
- Treating shock vs. surviving shock
- Monitoring response

Pharmacological Resuscitation

ELSEVIER

Resuscitation 54 (2002) 195–206

RESUSCITATION

www.elsevier.com/locate/resuscitation

cDNA array analysis of gene expression following hemorrhagic shock and resuscitation in rats

Hasan B. Alam [a,b,*,1], Svetlana Stegalkina [a,1], Peter Rhee [a,c], Elena Koustova [a]

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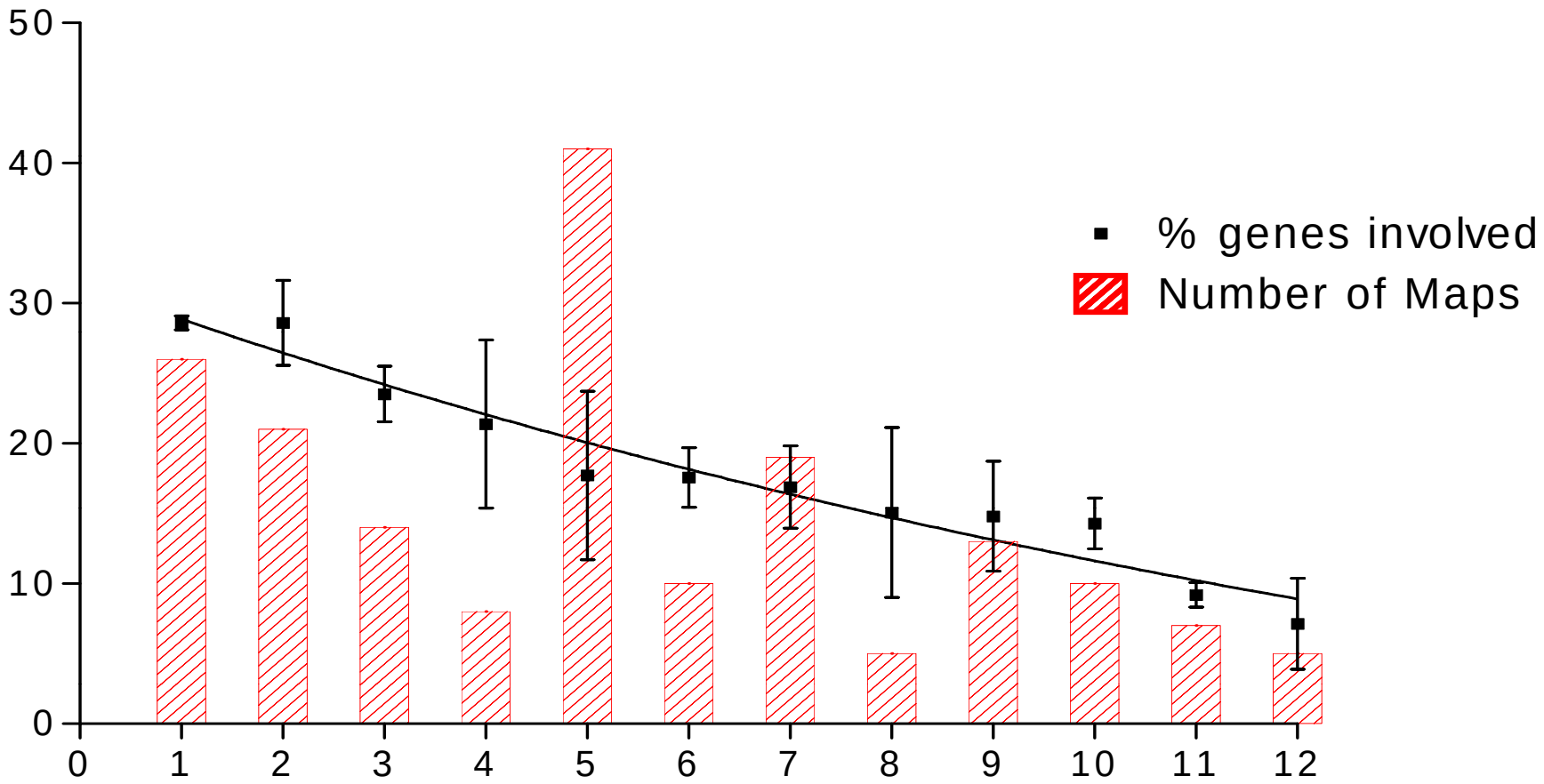
[c] The Naval Medical Center, San Diego, DC, USA

Identification of Expression Patterns Associated with Hemorrhage and Resuscitation: Integrated Approach to Data Analysis

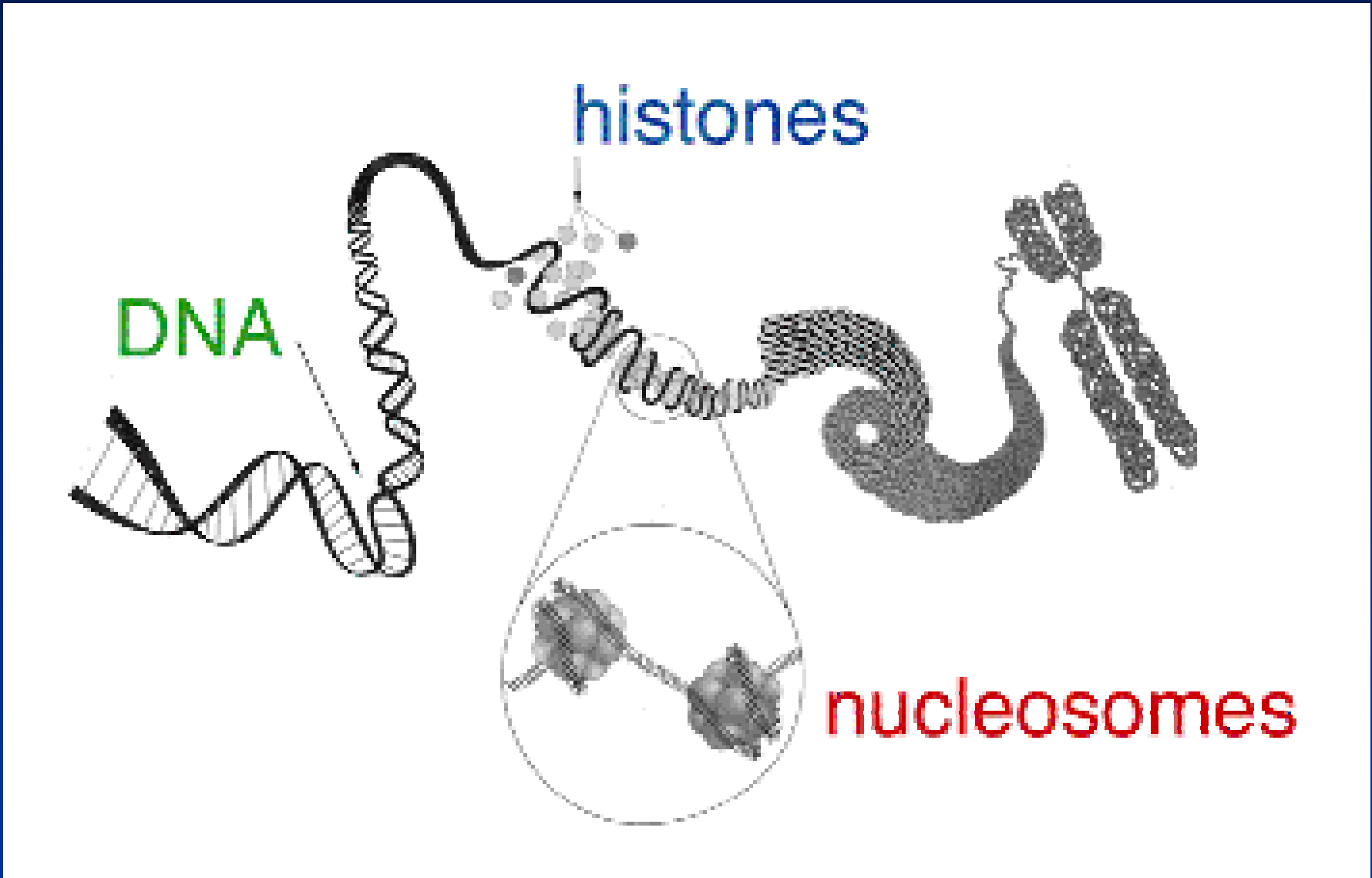
J Trauma. 2006;60:701–724.

Huazhen Chen, MD, Hasan B. Alam, MD, Racel Ireneo Luis C. Querol, MD, Peter Rhee, MD, Yongqing Li, MD, PhD, and Elena Koustova, PhD

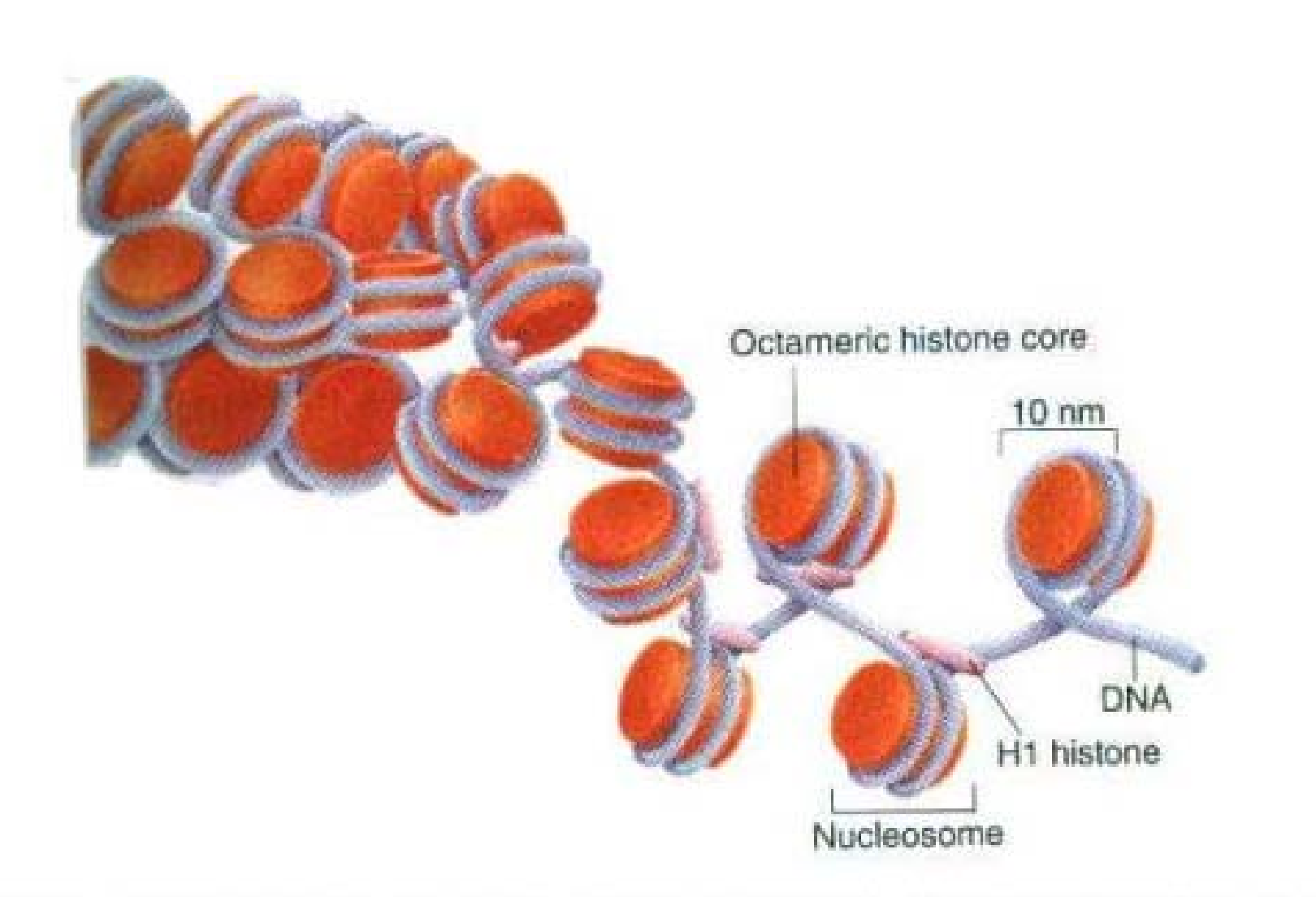


1. Transcription Factors
2. Apoptosis
3. Transcription Regulation
4. Metabolism Regulation
5. G-proteins
6. Cell Signalling
7. Growth Factors
8. Lipid Metabolism Regulation
9. Cytokines
10. Nucleotide Metabolism
11. Cell Adhesion
12. Carbohydrate Metabolism

Epigenetic regulation



Chromatin modulation



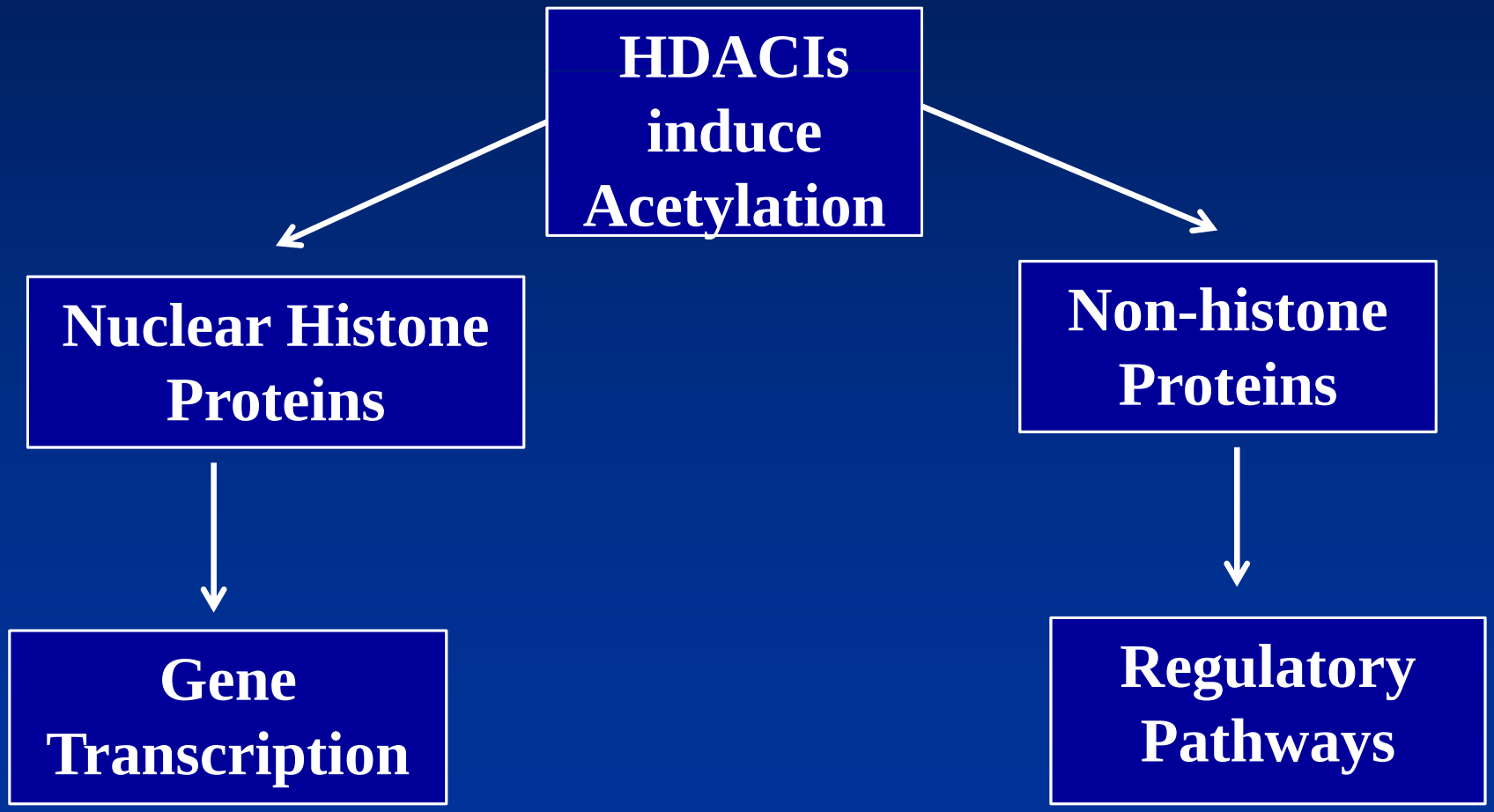
Acetylation

- Controlled by two enzyme systems:

Histone acetyltransferase (HAT) - ↑ transcription
Histone deacetylase (HDAC) - ↓ transcription

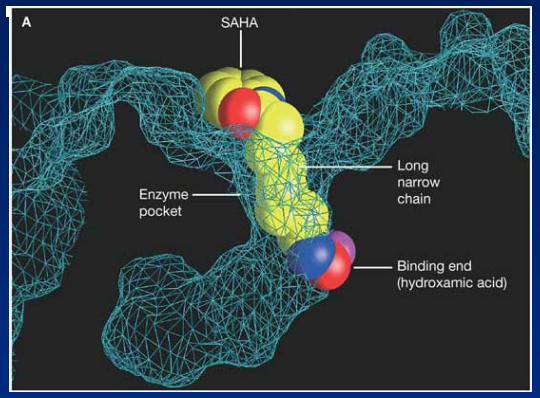
- Histone Deacetylase Inhibitors (HDACI)
  - Result in ↑ acetylation and ↑ transcription

HDAC Inhibitors



HDACI in clinical use

- **<u>Suberoylanilide hydroxamic acid</u> (SAHA)**
  - **Vorinostat (Zolinza™), Merck & Co., Inc**
  - **FDA approved Oct 2006**
    - **For cancer treatment (CTCL)**
    - **>40 ongoing clinical trials**



- **<u>Valproic acid</u> (VPA)**
  - **Depakote® or Depakene®, Abbott Pharmaceuticals, Ltd.**
  - **FDA approved Feb 1978**
    - **For seizure and mood disorders**
    - **Ongoing clinical trials for cancer treatment**
    - **Generic available**

Creating a "pro-survival phenotype"

Cardiac histones are substrates of histone deacetylase activity in hemorrhagic shock and resuscitation

(Surgery 2006;139:365-76.)

Tom Lin, MD, Hasan B. Alam, MD, Huazhen Chen, MD, Joy Britten-Webb, BS, Peter Rhee, MD, MPH, John Kirkpatrick, MD, *and* Elena Koustova, PhD, *Bethesda, Md, Washington, DC, and Boston, Mass*

Histone deacetylase as therapeutic target in a rodent model of hemorrhagic shock: Effect of different resuscitation strategies on lung and liver

(Surgery 2007;141:784-94.)

Tom Lin, MD, MPH,[a,b] Huazhen Chen, MD,[b] Elena Koustova, PhD,[b] Elizabeth A. Sailhamer, MD,[c] Yongqing Li, MD, PhD,[c] Christian Shults, MD,[a] Baoling Liu, MD,[c] Peter Rhee, MD, MPH,[b] John Kirkpatrick, MD,[a,b] *and* Hasan B. Alam, MD,[b,c] *Washington, DC, Bethesda, Md, and Boston, Mass*

Impact of resuscitation strategies on the acetylation status of cardiac histones in a swine model of hemorrhage☆

(Resuscitation, 2008; Volume 76, Issue 2, Pages 299-310)

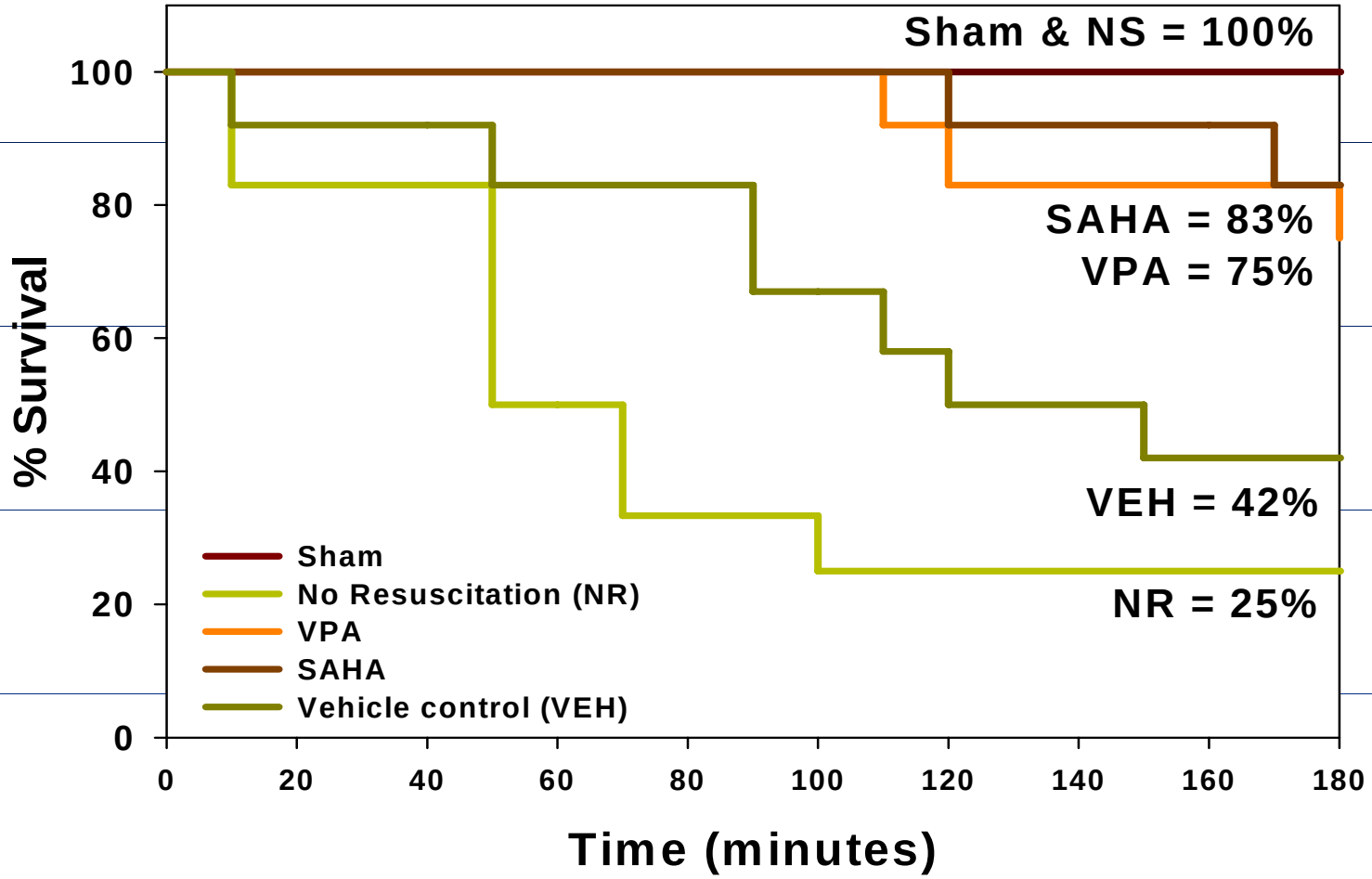
Hasan B. Alam[a,c,*], Christian Shults[b,c], Naresh Ahuja[a,b], Eduardo C. Ayuste[a], Huazhen Chen[a], Elena Koustova[a], Elizabeth A. Sailhamer[c], Yongqing Li[c], Baoling Liu[c], Marc de Moya[c], George C. Velmahos[c]

VALPROIC ACID PREVENTS HEMORRHAGE-ASSOCIATED LETHALITY AND AFFECTS THE ACETYLATION PATTERN OF CARDIAC HISTONES

SHOCK, Vol. 25, No. 4, pp. 395–401, 2006

Earl Gonzales, Huazhen Chen, Richard Munuve, Tina Mehrani, Joy Britten-Webb, Amal Nadel, Hasan B. Alam, David Wherry, David Burris, and Elena Koustova

HDACI treatment improves survival



The Journal of TRAUMA® *Injury, Infection, and Critical Care*

Surviving Blood Loss Without Fluid Resuscitation

Christian Shultz, MD, Elizabeth A. Sailhamer, MD, Yongqing Li, MD, PhD, Baoling Liu, MD, Malek Tabbara, MD, Muhammad Umar Butt, MD, Fahad Shuja, MD, Marc deMoya, MD, George Velmahos, MD, and Hasan B. Alam, MD

J Trauma 2008;64:629-640

Is acetylation the new phosphorylation?

EMBO J 2000

The Journal of TRAUMA® Injury, Infection, and Critical Care

Prevention of Hypoxia-Induced Neuronal Apoptosis Through Histone Deacetylase Inhibition

Yongqing Li, MD, PhD, Zengqiang Yuan, MD, PhD, Baoling Liu, MD, Elizabeth A. Sailhamer, MD, Christian Shults, MD, George C. Velmahos, MD, Marc deMoya, MD, and Hasan B. Alam, MD

Acetylation: a novel method for modulation of the immune response following trauma/hemorrhage and inflammatory second hit in animals and humans

Elizabeth A. Sailhamer, MD,[a] Yongqing Li, MD, PhD,[a] Eleanor J. Smith,[a] Fahad Shuja, MD,[a] Christian Shults, MD,[a,c] Baoling Liu, MD,[a] Chad Soupir, MD,[b] Marc deMoya, MD,[a] George Velmahos, MD,[a] *and* Hasan B. Alam, MD,[a] *Boston, Mass and Washington, DC*

Cell protective mechanism of valproic acid in lethal hemorrhagic shock

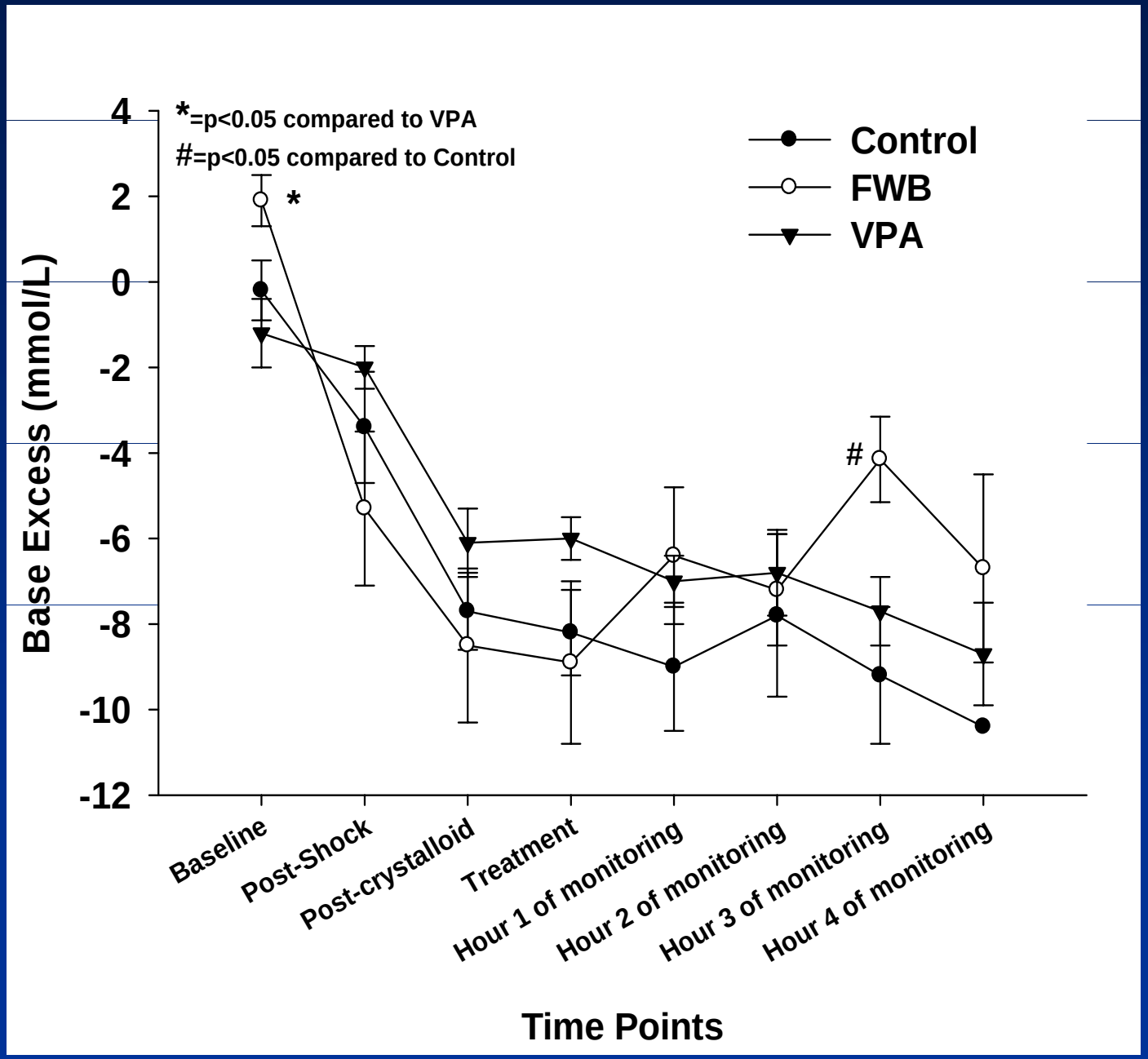
Yongqing Li, MD, PhD,[1] Baoling Liu, MD,[1] Elizabeth A. Sailhamer, MD,[1] Zengqiang Yuan, MD, PhD,[2] Christian Shults, MD,[1,3] George C. Velmahos, MD,[1] Marc deMoya, MD,[1] Azad Bonni, PhD,[2] *and* Hasan B. Alam, MD,[1] *Boston, Mass, Washington, DC*

Surviving blood loss without blood transfusion in a swine poly-trauma model

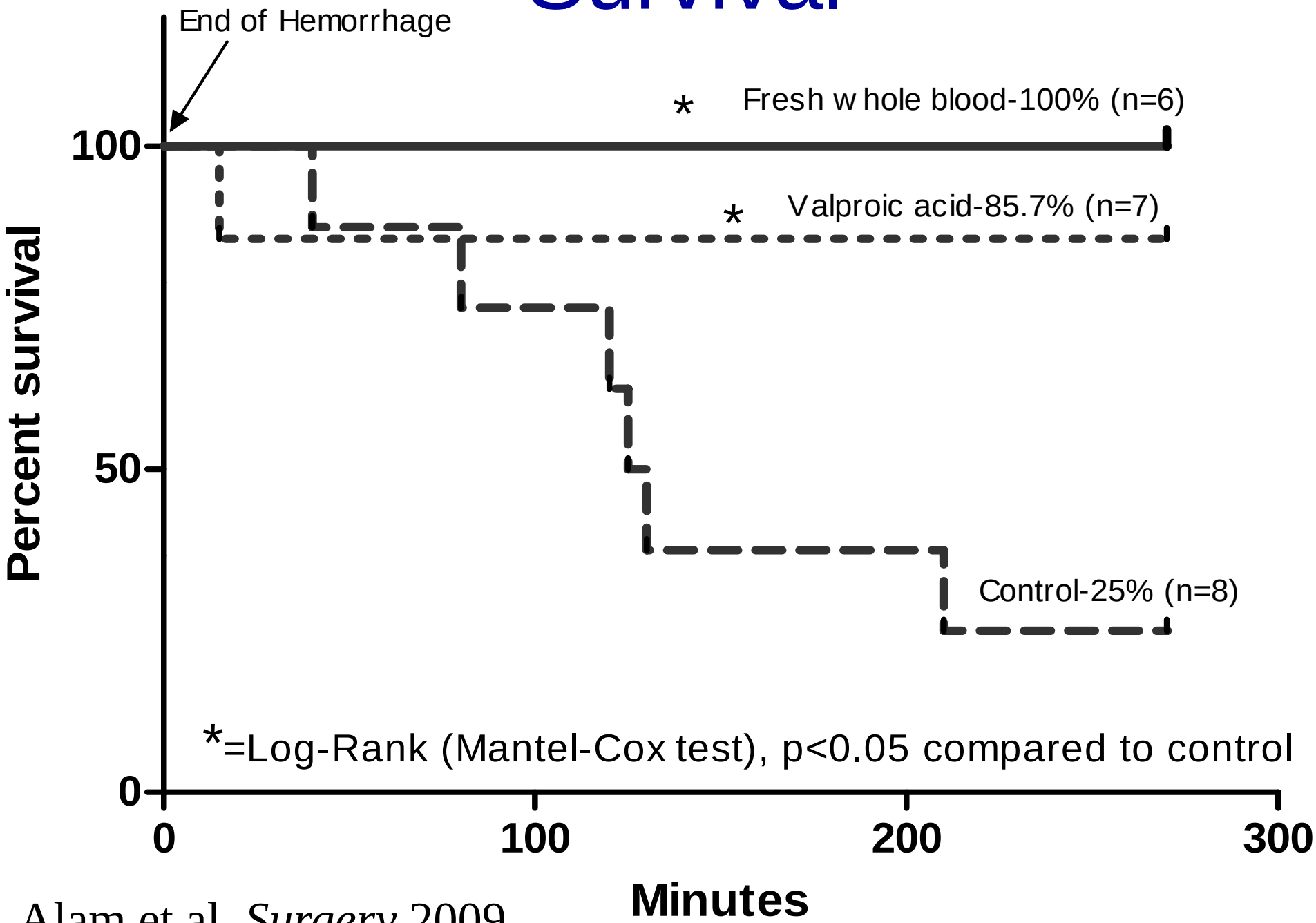
Hasan B. Alam, MD,* Fahad Shuja, MD,* Muhammad U. Butt, MD, Michael Duggan, DVM, Yongqing Li, MD, PhD, Nikolaos Zacharias, MD, Eugene Y. Fukudome, MD, Baoling Liu, MD, Marc deMoya, MD, *and* George C. Velmahos, MD, *Boston, Massachusetts*

Surgery 2009

Base excess

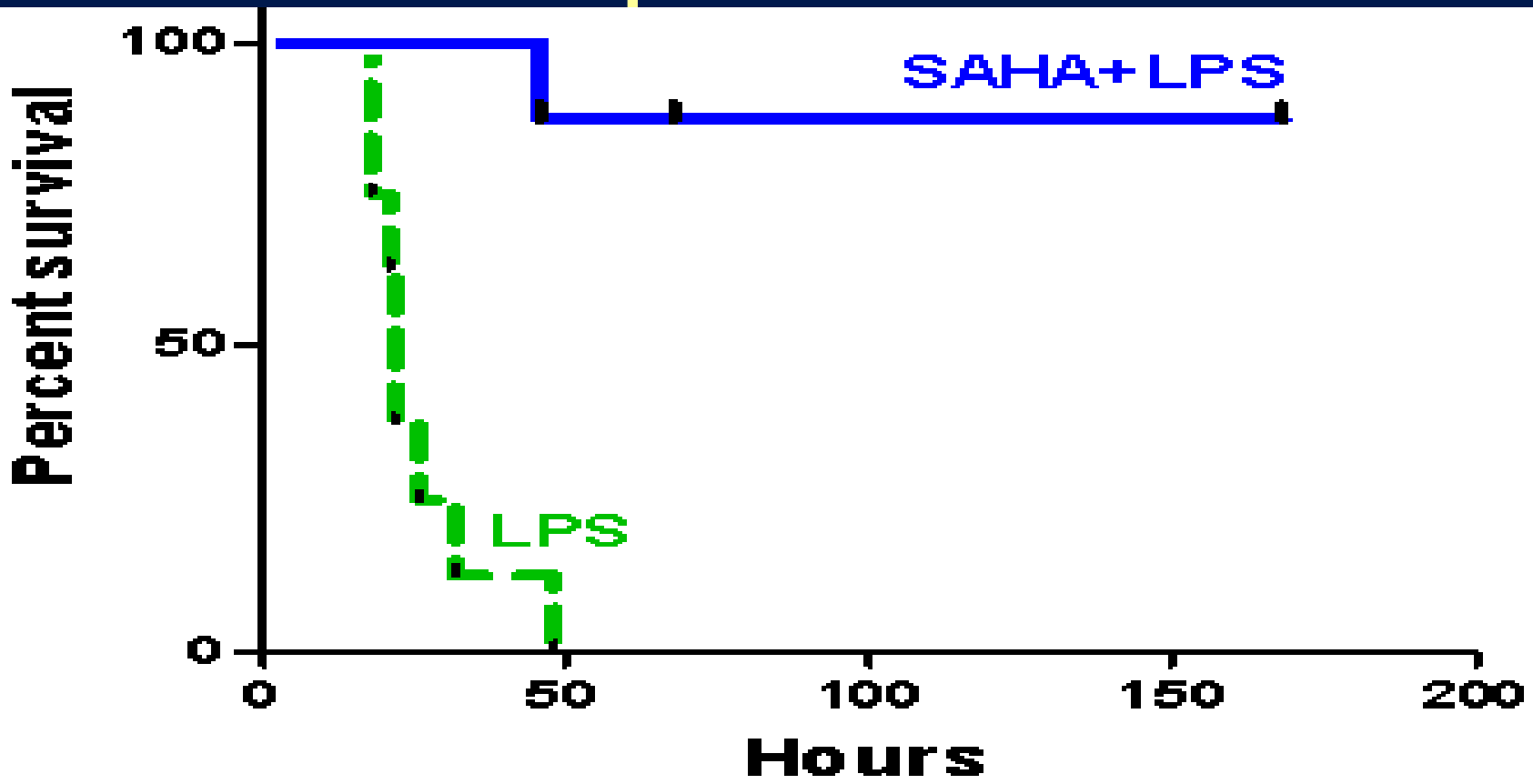

*=p<0.05 compared to VPA
#=p<0.05 compared to Control
Control
FWB
VPA
Base Excess (mmol/L)
4
2
0
-2
-4
-6
-8
-10
-12
*
#
Baseline
Post-Shock
Post-crystalloid
Treatment
Hour 1 of monitoring
Hour 2 of monitoring
Hour 3 of monitoring
Hour 4 of monitoring
Time Points

Survival



Alam et al. *Surgery* 2009

Septic Shock



PROTECTIVE EFFECT OF SUBEROYLANILIDE HYDROXAMIC ACID AGAINST LPS-INDUCED SEPTIC SHOCK IN RODENTS

Yongqing Li,* Baoling Liu,* Hang Zhao,† Eugene Y. Fukudome,* Xiaobo Zhang,‡ Tareq Kheirbek,* Robert Finkelstein,* George Velmahos,* Marc deMoya,* Charles A. Hales,† and Hasan B. Alam*

Shock 2009 March [E Pub ahead of print]

Biomarkers

- Diagnosis, treatment, monitoring and prognosis
- Limitations of traditional biomarkers
- Monitoring of tissue perfusion vs. cellular survival
- Desirable qualities

Methods

Discovery of circulating biomarkers

- Serum: BL (baseline), HS and VPA treatment in a rat model
- Determination of protein profile patterns: surface-enhanced desorption / ionization time-of-flight mass spectrometry (SELDI-TOF MS)
- Identification of biomarkers: isobaric tag labeling for relative quantitation (iTRAQ)
- Validation of a biomarker: Western blotting

Identification of a Novel Potential Biomarker in a Model of Hemorrhagic Shock and Valproic Acid Treatment[1]

Yongqing Li, M.D., Ph.D.,[*,3] Baoling Liu, M.D.,[*,3] Simon T. Dillon, M.D.,[†] Eugene Y. Fukudome, M.D.,[*] Tareq Kheirbek, M.D.,[*] George Velmahos, M.D., Ph.D.,[*] Marc deMoya, M.D.,[*] Towia A. Libermann, M.D.,[†] and Hasan B. Alam, M.D.[*,2]

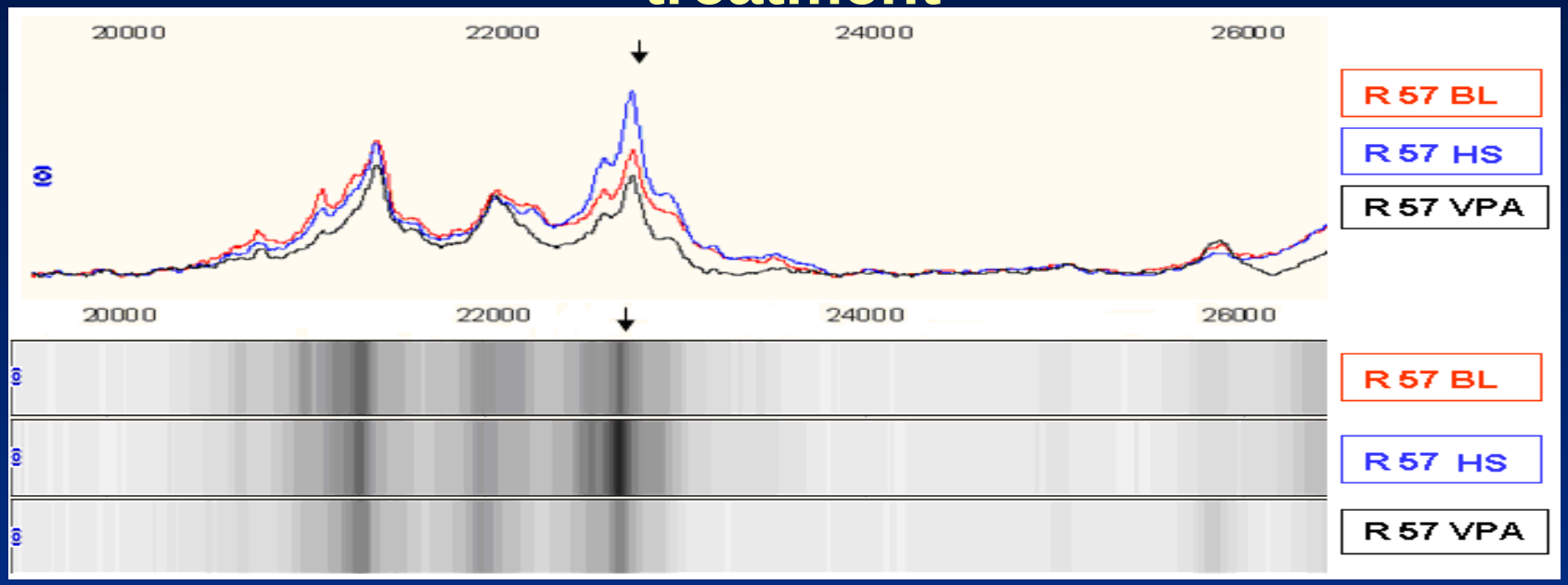
J Surg Research 2009

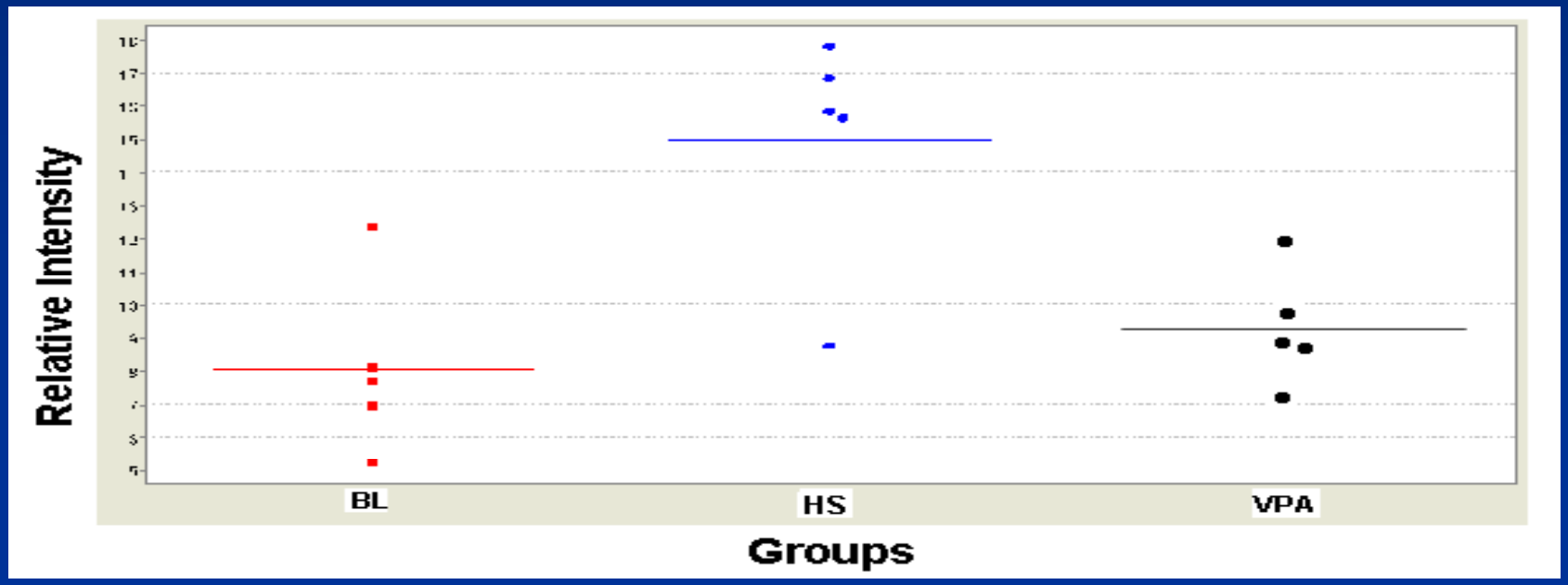
Biomarkers

Serum Biomarker Levels following hemorrhage and VPA Treatment

Accession number	Protein name	Function	Ratio (BL/EH)	Ratio (VPA/EH)
Q63400|CLD 3_RAT	Claudin-3	Plays a major role in tight junction-specific obliteration of the intercellular space, through calcium-independent cell-adhesion activity.	0.324	0.425
P04638|APO A2_RAT	Apolipoprotein A2	Instrumental in promoting efflux of cholesterol from extra-hepatic tissue to the liver.	0.72	0.44
P70505|ADA M1_RAT	Disintegrin and metalloproteinase domain-containing protein 1 precursor - Rattus norvegicus (Rat)	May be involved in sperm-egg fusion.	0.779	0.724
P00762|TR Y1_RAT	Anionic trypsin-1 precursor - Rattus norvegicus (Rat)	A protease. Preferential cleavage: Arg-/Xaa, Lys-/-Xaa.	0.629	0.335
P08649|CO4 _RAT	Complement C4 precursor [Contains: Complement C4 beta chain; Complement C4 alpha chain; C4a anaphylatoxin; Complement C4 gamma chain] - Rattus norvegicus (Rat)	C4a anaphylatoxin is a mediator of local inflammatory processor that induces the contraction of smooth muscle, increases vascular permeability and causes histamine release from mast cells and basophilic leukocytes.	0.869	0.813
P01015|ANG T_RAT	Angiotensinogen precursor - Rattus norvegicus (Rat)	In response to lowered blood pressure, the enzyme rennin cleaves angiotensin-1, from angiotensinogen. ACE (angiotensin converting enzyme) then removes a dipeptide to yield physiologically active peptide angiotensin-2, the most potent pressor substance known, which helps regulate volume and mineral balance of body fluids.	1.24	1.33
O54766|ZP 1_RAT	Zona pellucida sperm-binding protein 1 precursor - Rattus norvegicus (Rat)	Mediates species-specific sperm binding, induction of the acrosome reaction and prevents post-fertilization polyspermy	0.615	0.273
P06882|TH YG_RAT	Thyroglobulin precursor - Rattus norvegicus (Rat)	Precursor of the iodinated thyroid hormones thyroxine (T4) and triiodothyronine (T3).	1.27	1.90
O35276|NR P2_RAT	Neuropilin-2 precursor - Rattus norvegicus (Rat)	High affinity receptor for semaphorins 3C, 3F, VEGF-165 and VEGF-145 isoforms of VEGF, and the PLGF-2 isoform of PGF.	0.538	0.612
P70545|S13 A2_RAT	Solute carrier family 13 member 2 - Rattus norvegicus (Rat)	Membrane protein. Cotransport of sodium ions and dicarboxylates such as succinate and citrate.	0.665	0.558
Q9Z0W0|G LP2R_RAT	Glucagon-like peptide 2 receptor precursor - Rattus norvegicus (Rat)	The activity of this receptor is mediated by G proteins that activate adenylyl cyclase.	0.776	0.691
Q7TSY2|L HPL4_RAT	Lipoma HMGIC fusion partner-like protein 4 - Rattus norvegicus (Rat)	Membrane protein. Function unknown.	0.787	0.695

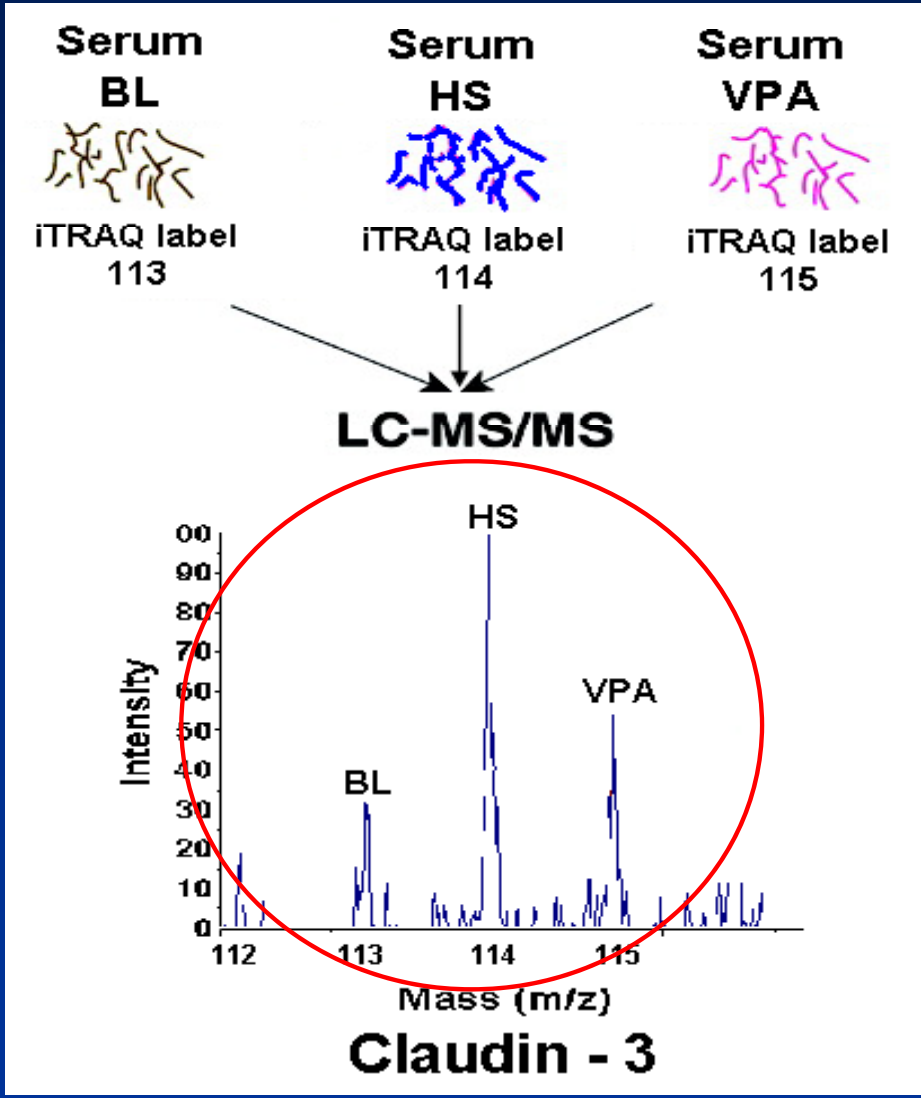
Determination of protein profile patterns: Application of SELDI following hemorrhage and VPA treatment



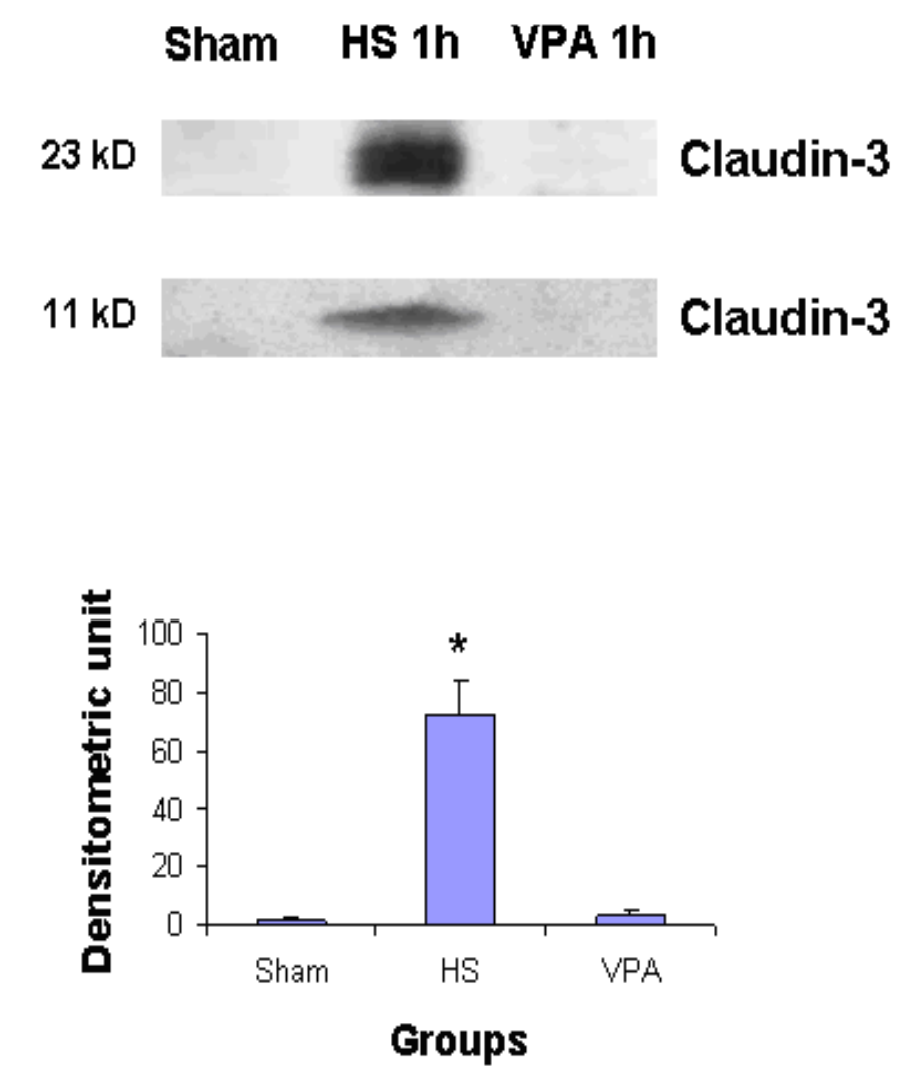


Identification, quantification and validation of biomarker claudin-3 protein

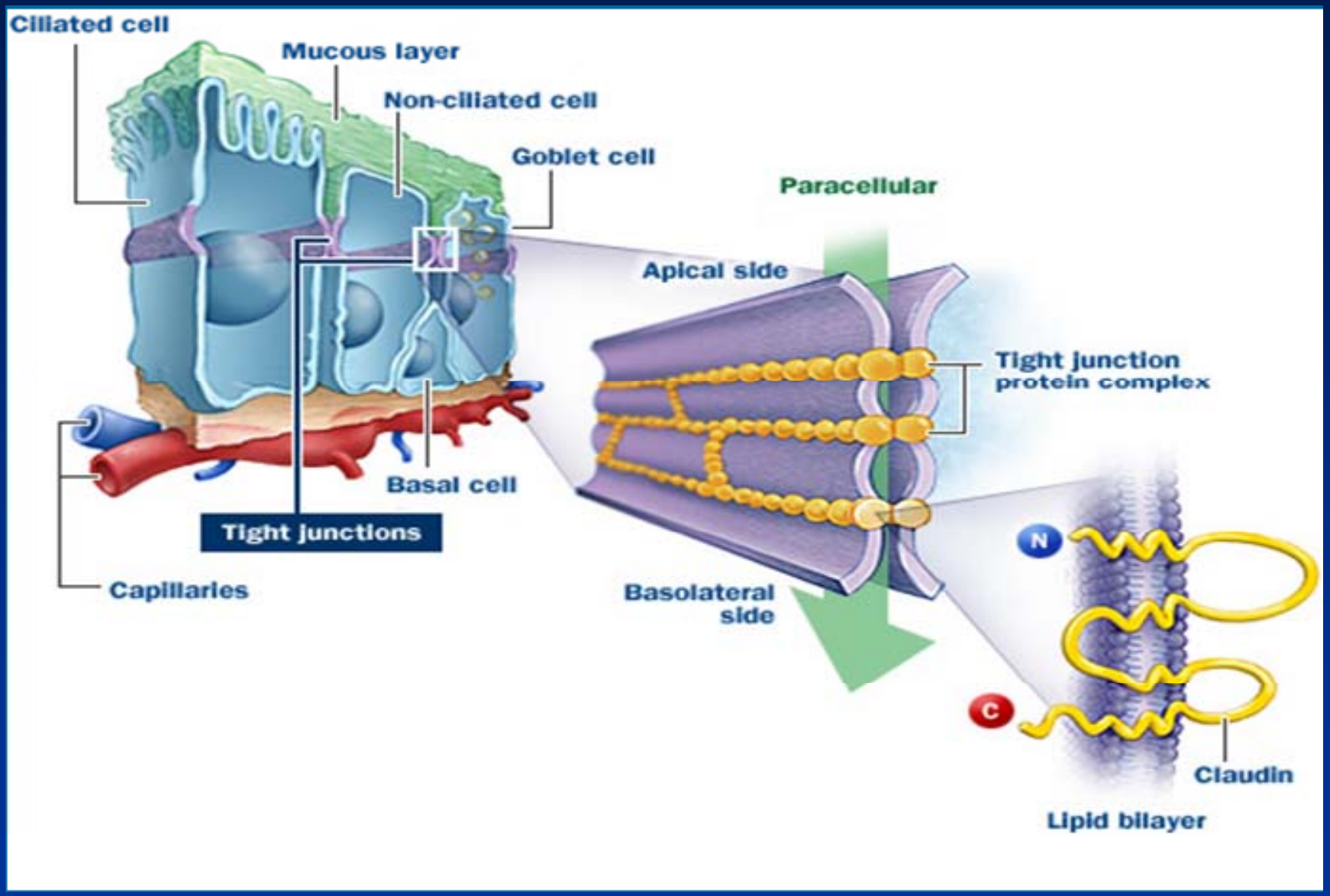
iTRAQ Identification and Quantification



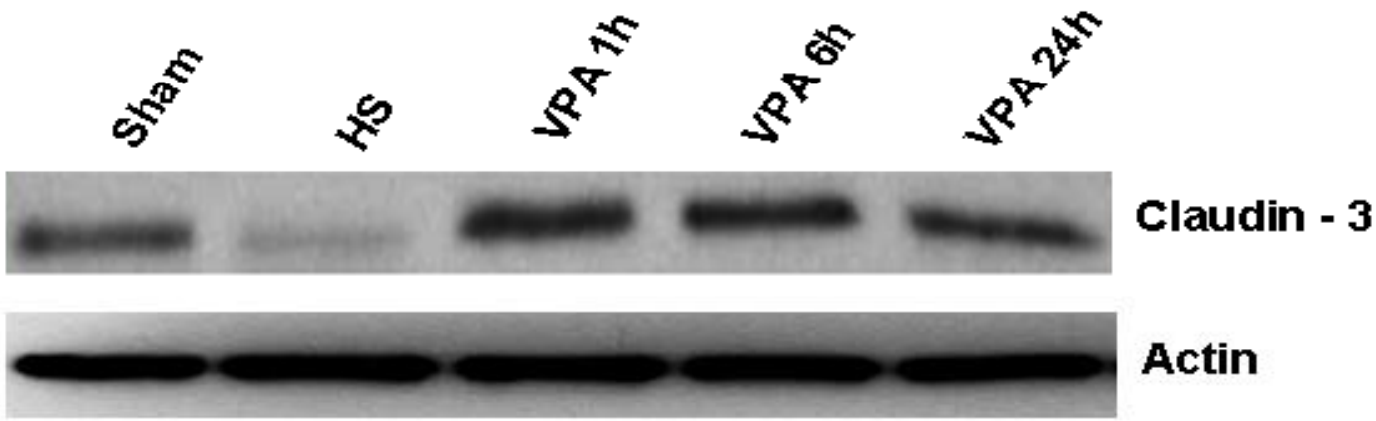
Western Blot Validation

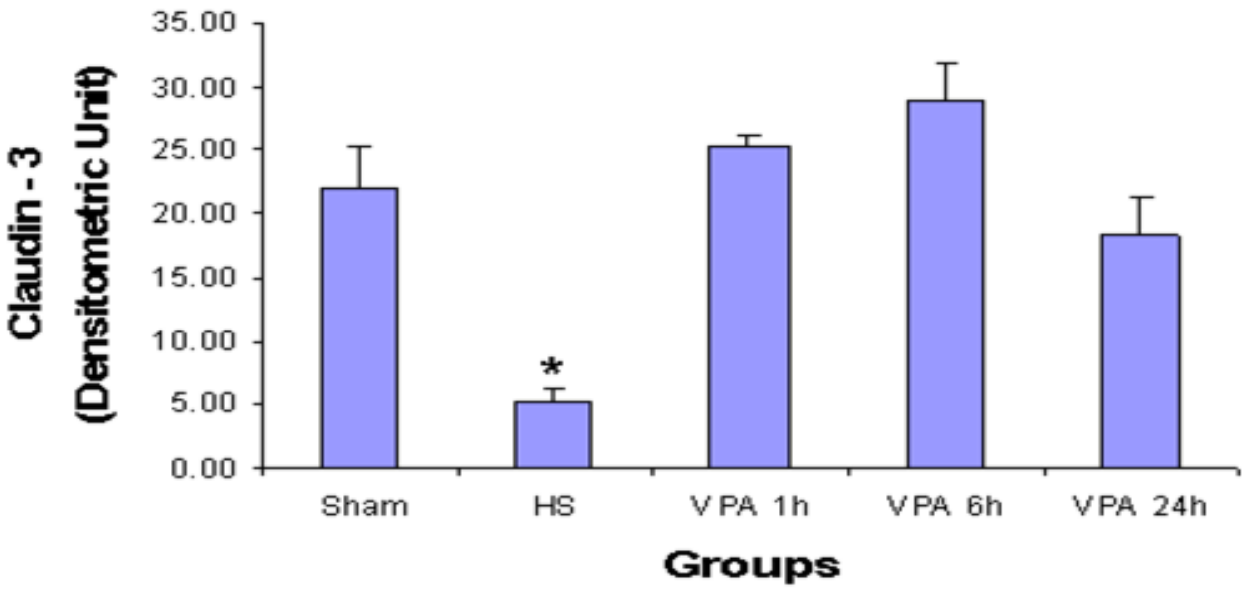


Claudin-3 and tight junction in intestinal epithelial cells

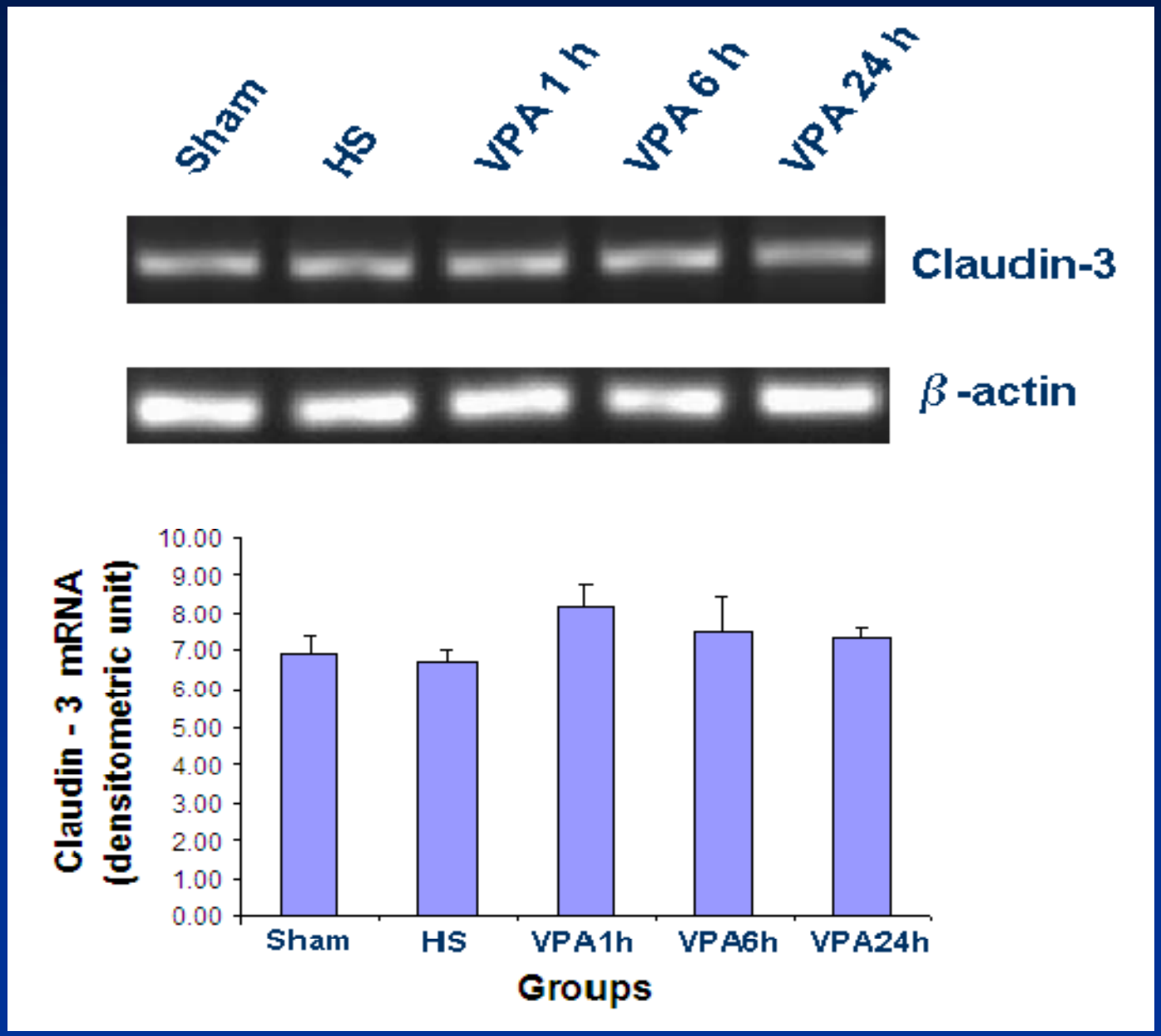


Response of intestinal claudin-3 to hemorrhage and VPA treatment

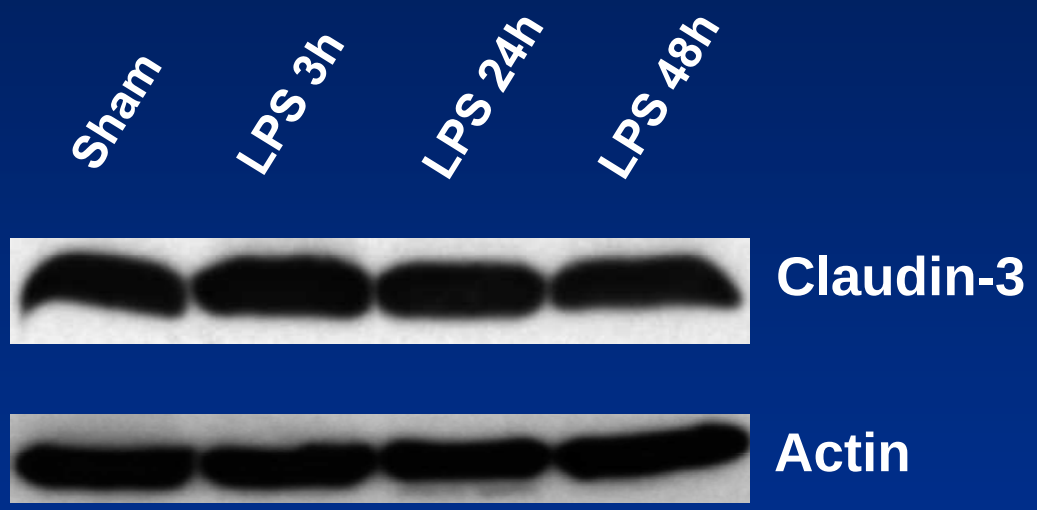




Expression of Intestinal claudin-3 mRNA



Expression of claudin-3 protein in intestine after LPS-induced septic shock



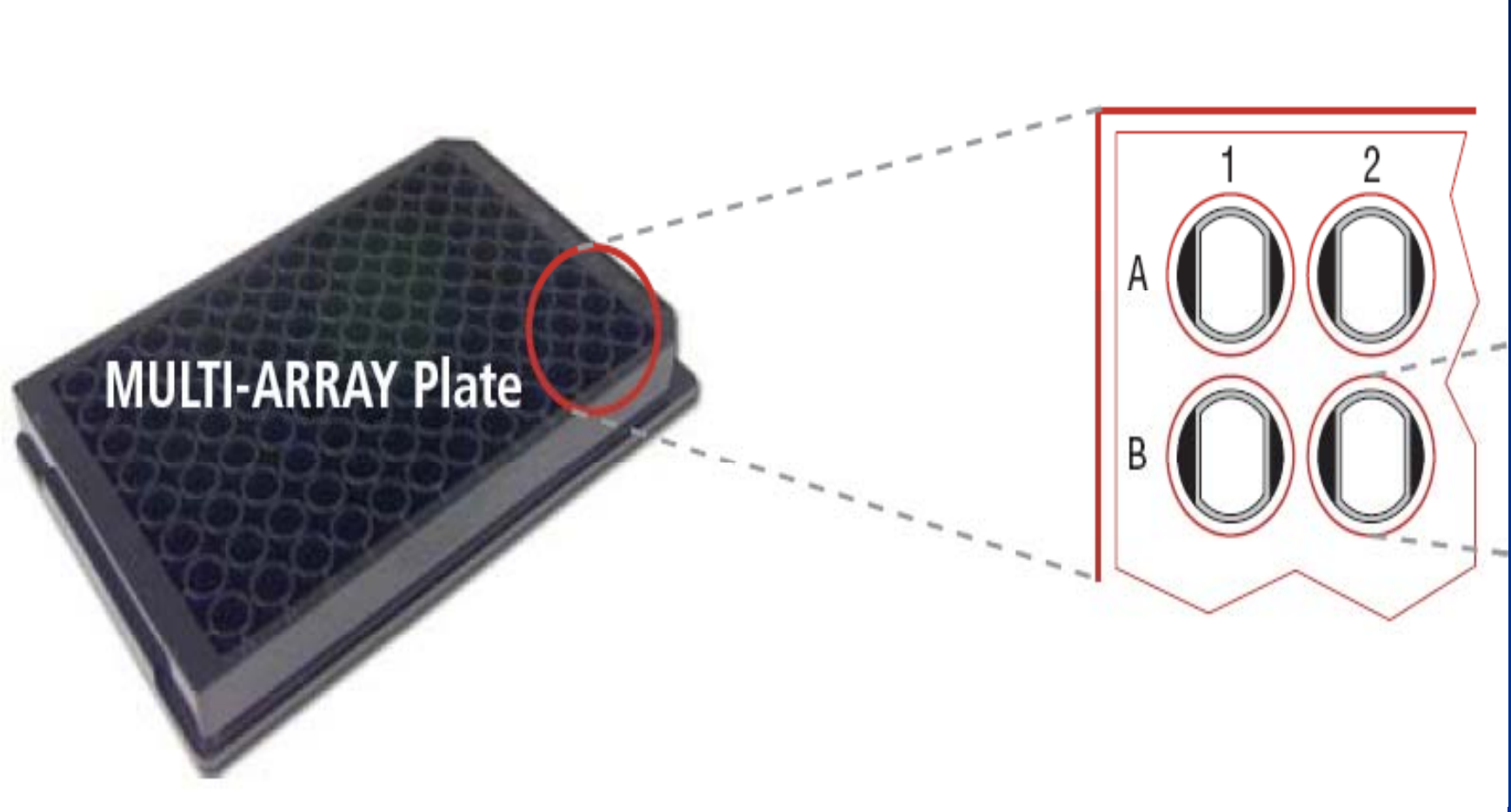
Changes specific to hemorrhage

Conclusions

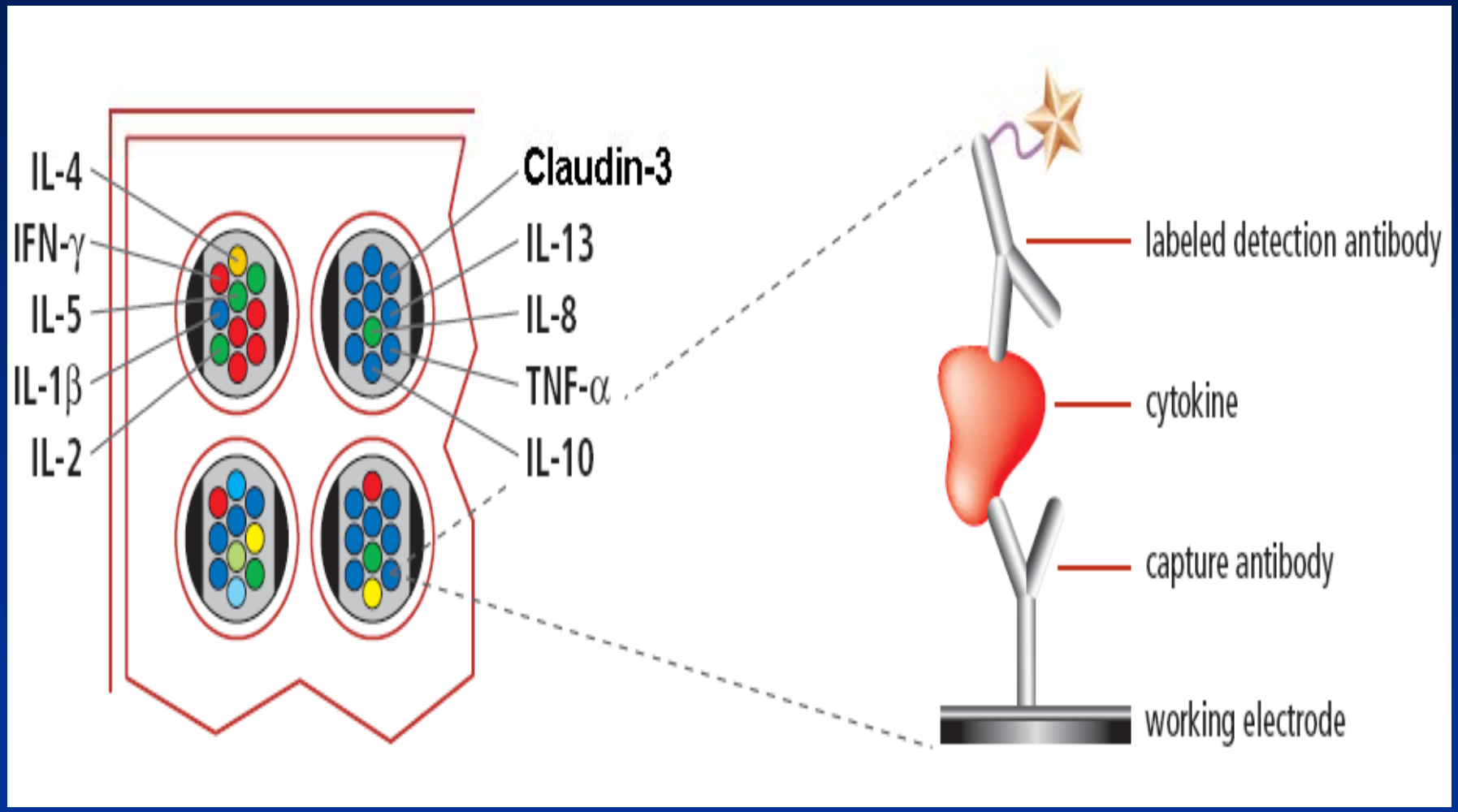
- HDACI treatment restores acetylation balance and improves survival
- Hemorrhage causes an acute rise in serum biomarkers, such as claudin-3
- With a concurrent decrease in intestinal claudin-3
- HDACI treatment prevents these changes
- Correlates with survival

Next Steps:
Clinical correlation using high throughput, rapid assay

Electrochemiluminescence Detection for Screening of Serum Biomarkers in Trauma Patients



Selected Biomarkers for Screening Trauma Patients



Acknowledgments

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Massachusetts General Hospital

- *Yongqing Li, MD, PhD*
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- *Elizabeth Sailhamer, MD*
- *Marc A. deMoya, MD*
- *George C. Velmahos, MD*
- *David King, MD*

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