Diseases and Discoveries of the Urothelium
Workshop 11
Monday 23 August 2010, 09:00 – 12:00

Time	Time	Topic	Speaker
09.00	09.10	Introduction	Ricardo Saban
09:10	09:50	Translational Animal Models	Firouz Daneshgari
09:50	10:30	The urothelium	Lori Birder
10:30	11:00	Break	
11:00	11:30	Bladder sensory desensitisation decreases urinary urgency	Francisco Cruz
11:30	12:00	Neuropilins (NRP1 and NRP2).	Ricardo Saban

Aims of course/workshop

In this workshop, we will discuss: **1)** An interdisciplinary approach to create new translational animal models of Lower Urinary Tract Dysfunction; **2)** LUTD-induced augmented release of urothelial-derived transmitters such as ATP or acetylcholine and their impact in bladder nerve excitability; **3)** Bladder desensitization by intravesical resiniferatoxin RTX, as treatment for refractory detrusor overactivity; **4)** New pharmacological targets: VEGF receptors and neuropilins that are highly expressed in the human and mouse urothelium, downregulated
in patients with IC/PBS, and up-regulated by intravesical BCG therapy.

Educational Objectives

The present workshop is being presented by leaders in the field of translational LUTD. Current treatment modalities for LUTD only relieve the storage or voiding bladder symptoms. Lack of a serious treatment or preventive strategies for LUTD over the past several decades has led to development of new translational animal models, a desirable step in the hierarchy of research to jump start translational research. In this workshop, we will discuss several breakthroughs: **1)** interdisciplinary approach among investigators in immunology, urology and neuroscience, to create mouse models of LUTD. Further, we will discuss the process of LUT phenotyping and characterization; and dissemination of these models to the scientific community. We will also approach the: **2)** LUTD-induced disruption of urothelium-nerve communication, **3)** Desensitization of vanilloid receptors as treatment of LUTD **4)** the role of urothelial neuropilins and VEGF receptors in LUTD.

Animal Models of Lower Urinary Tract Dysfunction

Firouz Daneshgari, M.D.
Lester Persky Professor & Chairman
Urology Institute
University Hospitals of Case Medical Center
Case Western Reserve University

Lower Urinary Tract Dysfunction

- Diseases:
  - Among the most common diseases affecting both genders and at ALL ages
- Prevalence
  - Up to 50% of Women
  - Up to 40% of Men
  - Up to 15% of children

Lower Urinary Tract Dysfunction

- Women:
  - Pelvic Organ Prolapse
  - Urinary Incontinence: SUI, OAB
  - Interstitial Cystitis
  - Pelvic Pain
  - Neurogenic Bladder: Multiple Sclerosis
  - UTI
- Men:
  - BPH/LUTS
  - Interstitial Cystitis
  - Urinary Incontinence- OAB
  - Neurogenic Bladder: SCI, Parkinson, CVA,

Lower Urinary Tract Dysfunction

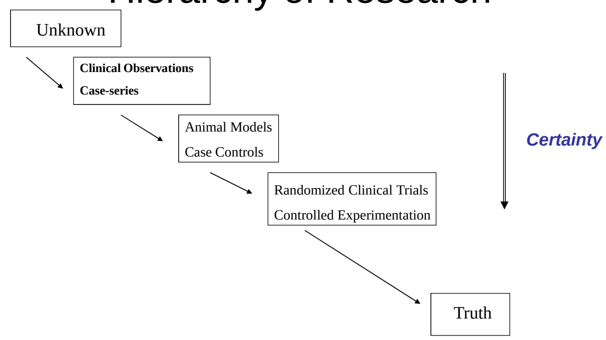
- Children:
  - UTIs
  - Voiding Dysfunction
  - Bed Wetting
  - Neurogenic Bladder
  - Vesicoureteral reflux

LUTD

- Quality of life diseases:
  - Difficult to quantify symptoms, signs
- Complex interaction between CNS, Spine and LUT organs
- Plasticity: neuroplasticity; threshold sensitization= adaptation to insult= moving target

Path of Discovery in Clinical Sciences

Hierarchy of Research



Animal Models

- Evolutionary Hierarchy:
  - Larger to smaller: Closer- Further from human:
  - POP in Monkeys vs Mice!
- Feasibility and Economics Hierarchy:
  - Diabetes in pigs vs in mice
- Examples of other diseases:

Animal Models of LUTD

- Naturally occurring:
  - Feline I.C.
  - SUI in female dogs
- Induced:
  - Similar Pathophysiology
    - Destruction of Pancreatic Beta cell- Type I DM
    - Vaginal Distension for SUI
  - Similar Phenotype:
    - POP in Loxl-1
- Knock out-transgene Technology

Aims of AMLUTD

- Phenotype
- Pathophysiology
- Phenotype+Pathophysiology:

Available AMLUTD

- POP
- SUI
- Cystitis
- I.C.
- NGB:
  - Parkinson
  - M.S.
  - SCI
- Prostatitis
- Sling

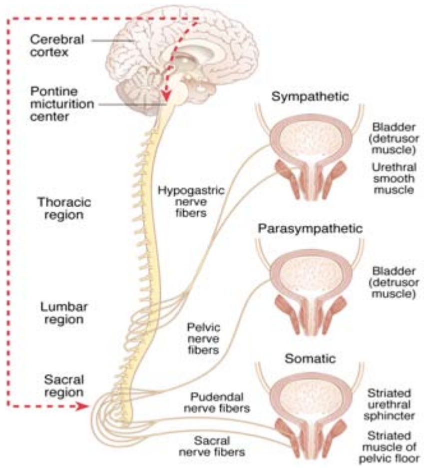
Phenotype Validation

- Challenges:
  - Incompatibilities with human symptoms/signs
  - Unknown human phenotype
- Opportunities:
  - » Provide opportunity to re-examine Human phenotype
  - » Development of devices/diagnostic tools
- Solution:
  - Start with a table of comparison: human vs model phenotype

LUT Phenotype tools

- 24 micturition (Bladder Diary)
- CMG (urodynamics)
- Anatomical comparisons (mPOPQ)
- Estimation of pain
- Estimation of behavior changes
- Biological samples (urine, serum, tissue, DNA, proteomics)

Innervation and Function of the Lower Urinary Tract

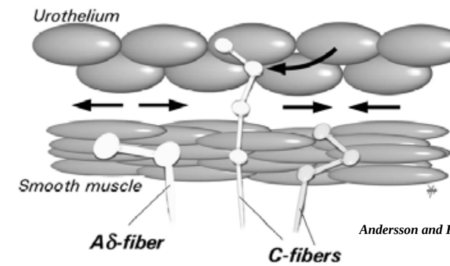


The lower urinary tract (LUT) has 2 main functions – storage and periodic elimination of urine

These functions are regulated by complex neural control systems

Release of factors from the urothelium during bladder stretch may activate suburothelial nerves---and play a role in voiding

Alterations in release may also contribute to bladder instability, hyperactivity and altered bladder sensation



Andersson and Hedlund, Urology, 60:13-21, 2002.

Changes in the urothelium appear now to be an important factor in a number of bladder syndromes

The urothelium: What do we know?

Release of mediators

Umbrella Cells

"Sensor" Function

prostacyclin

NGF

Nitric oxide

cytokines

Uroplakins

"Neuronal-like" properties

Tight Junctions

TRPV1

Barrier Function

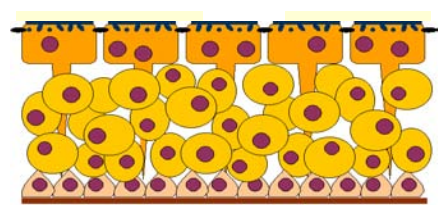
Acetylcholine

Permeability

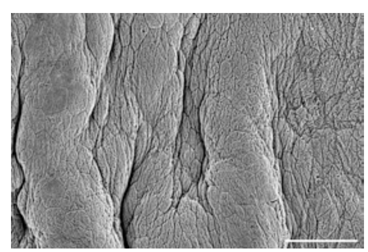
ATP

Urothelium (UT):

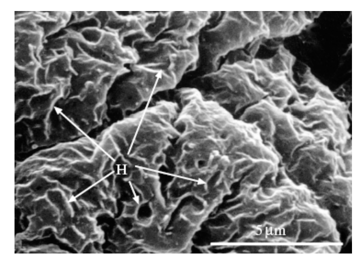
Multilayered structure (transitional epithelium) consisting of apical, intermediate and basal cells.



The "outermost" umbrella cell layer is composed of large cells (250 μm in diameter). These are the largest epithelial cells in the body.

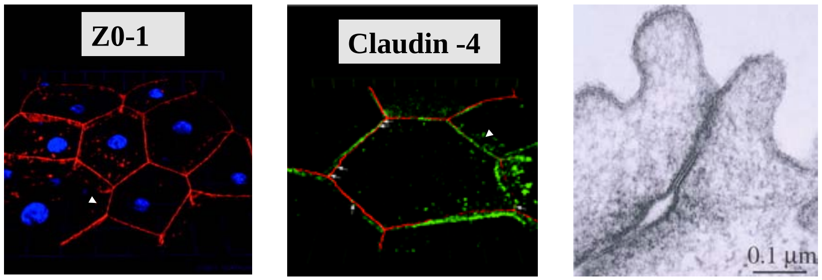


Apical Urothelial Cells



The barrier function of the epithelium is defined by a combination of various tight-junction proteins

The “claudins” (4, 8 and 12) are associated with high resistance tight junctions and also may be important in cell adhesion



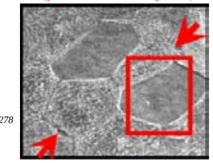
Archaya et al., American Journal of Physiology, 287:F305-318, 2004.

These may be important in various diseases such as bacterial cystitis, interstitial cystitis and spinal cord injury. Each of these conditions is characterized by a disruption of the urothelial barrier

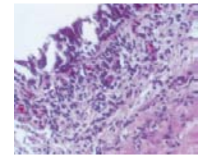
Mucosal Barrier may be disrupted in various diseases such as Painful Bladder Syndrome / Interstitial Cystitis **(*Bladder Pain Syndrome*)** ***and Spinal Cord Injury***

Cats with PBS/FIC
(feline interstitial cystitis)

Lavelle et al; AJP 278 F540-545, 2000.



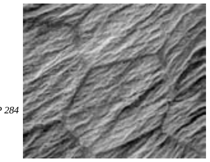
Humans with PBS/IC



Tomaszewski et al., Urology 57: 67-81, 2001

Control

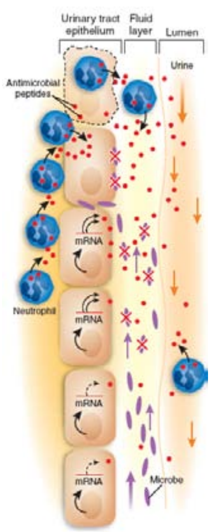
Apodaca et al., AJP 284 F966-976, 2003



2 hr SCI

The acute changes in epithelial structure/ function following SCI suggest an involvement of bladder nerves

When the barrier is compromised, it can result in passage of toxic substances into the underlying tissue resulting in urgency, frequency and pain during distension



The urothelial “barrier” can be altered by *infection*

There is interest in how pathogens (*Ecoli*) found in UTI can interact with urothelial cells and thereby infect the host

Rouschop et al., J Immunol 177:7225-32, 2006.

Garofalo et al., Infect Immunol 75: 52-60, 2007.

Ratliff TL. J Urology, 174:1150, 2005.

Slechta and Mulvey, Trends in Microbiology, 14:58-60, 2006.

More than just a barrier: The Urothelium Functions as a Primary Transducer of Physical and Chemical Stimuli

4 Lines of evidence lend support for involvement of the urothelium in visceral sensation:

(1) Expression of “neuronal-like” receptors/ion channels

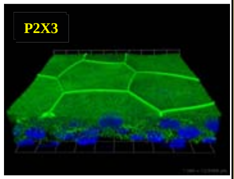
(2) Location in close proximity to bladder nerves

(3) Release of transmitters/mediators

(4) Plasticity in signaling functions induced by pathological conditions

Sensory Roles of the Urothelium

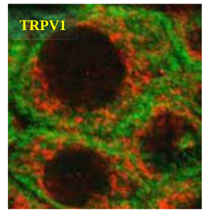
Urothelial cells express receptors/ion channels similar to mechanoreceptive and nociceptive afferent nerves



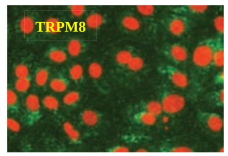
Wang et al JCI 115:2412-22,2005

Polymodal Properties of Urinary Bladder Urothelial Cells

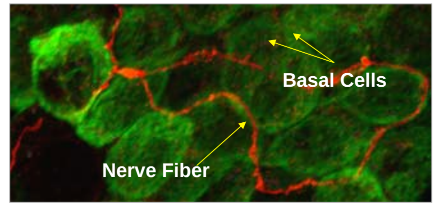
Sensor Function/Stimuli	Urothelial Sensor Molecules	Neuronal Sensor Molecules
ATP	P2X/P2Y	P2X/P2Y
Capsaicin/Resiniferatoxin	TRPV1	TRPV1
Heat	TRPV1; TRPV2; TRPV4	TRPV1; TRPV2; TRPV3; TRPV4
Cold	TRPM8; TRPA1	TRPM8; TRPA1
H^+	TRPV1; ?	TRPV1; ASIC; DRASIC
Osmolarity	In part TRPV4	In part TRPV4
Bradykinin	B1; B2	B1; B2
Acetylcholine	Nicotinic/muscarinic	Nicotinic/muscarinic
Norepinephrine	α/β subtypes	α/β subtypes
Nerve Growth Factor	p75/trkA	p75/trkA
Mechanosensitivity	Amiloride Sensitive Na^+ Channels	Amiloride Sensitive Na^+ Channels



Birder et al PNAS 98:13396-401, 2001



The location of bladder nerves in close proximity to the epithelium suggest potential for a chemical dialogue



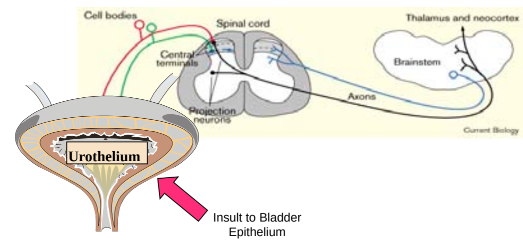
Birder et al., PNAS 98:13396-401, 2001.

Bladder afferents and efferents are located in close proximity to the urothelium

Sensitization of Afferents; Bladder Pain

Disruption of the barrier can lead to altered activation of bladder nerves

Associated with increased frequency/urge



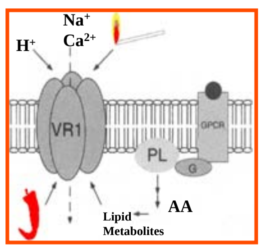
An example of a urothelial sensor molecule that is altered in bladder pathology is the Capsaicin Receptor, TRPV1, a cation channel expressed by afferent neurons of the 'pain' pathway.

TRPV1 is known to play a prominent role in nociception and in urinary bladder function

VR1 (TRPV1)

- **Calcium permeable, non-selective ion channel**
- **Activated by capsaicin, heat and protons**

Caterina et al., Nature 389: 816-824, 1997 and Nature 398:436-441, 1999.



Historical Perspective:

1919: Identification of capsaicin from hot peppers *(Nathan, J AM Chem Soc 41: 1115-1117).*

1955: Jancso observed nociceptive nerve endings were activated/inactivated by capsaicin *Jancso et al, Br J Pharm 1968; Nature 1977).*

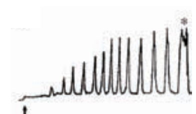
1989: Szallasi *(Szallasi and Blumberg, Neuroscience 1989)* showed specific binding sites for capsaicin in urinary bladder/urethra.

1997: Cloning of the capsaicin receptor: TRPV1 *(Caterina et al, Nature 289: 816-814, 1997).*

2006: TRPV1---integrator of thermal/noxious chemical stimuli---expressed in afferent fibers innervating the bladder.

Vanilloid Receptor and Detrusor Instability:

Capsaicin-sensitive (C type) fibers shown to be involved in detrusor hyperreflexia of spinal origin and in bladder pain processing *(de Groat et al., JANS 1990; Cheng et al., AJP 1999).*



SCI Cat

SCI Cat & Capsaicin treatment

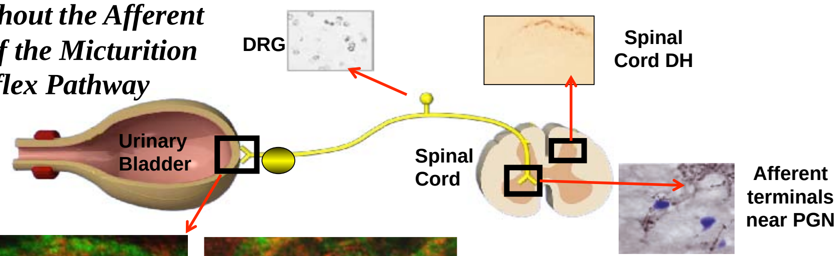
This provided the basis for intravesical administration of capsaicin or RTX in patients with bladder hyperreactivity resulting from a spinal cord injury (*Fowler; Urology 2000*) (and in patients with idiopathic detrusor instability).

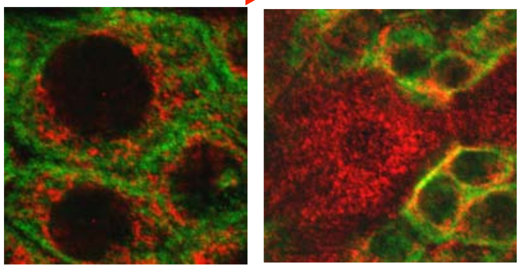
This treatment produces significant symptomatic relief and results in increased bladder capacity, decreased urinary frequency and urge incontinence episodes.

GOAL:

To explore the physiological significance of TRPV1 in the urinary bladder

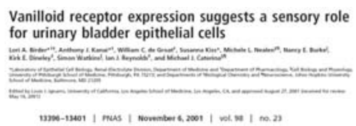
TRPV1 is Expressed Throughout the Afferent Limb of the Micturition Reflex Pathway





Basal Cells | **Apical Cells**

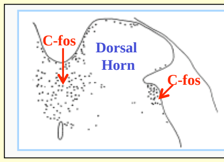
…..by the urothelial cells themselves


Vanilloid receptor expression suggests a sensory role for urinary bladder epithelial cells

13396–13401 | PNAS | November 6, 2001 | vol. 98 | no. 23

Mice lacking TRPV1 show loss-of-function bladder phenotypes

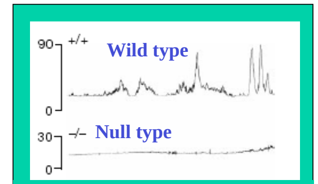
- **Increased Bladder Capacity**
- **Decreased Fos-response to bladder filling**



C-fos: marker for activation of second-order SC neurons (receiving input from bladder afferents)

These results suggests abnormal detection of bladder "stretch"

Mice lacking the P2X$_3$ ATP receptor subunit (expressed on bladder afferents) show abnormal reflex contractions/voiding behavior



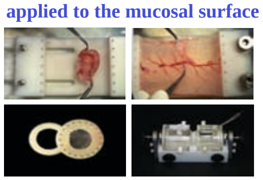
Knockout mice display reduction in pain responses & response to bladder filling

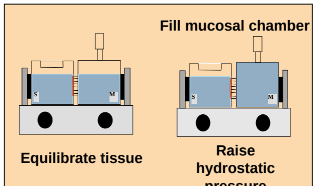
Cockayne et al., Nature 407:1011-1015, 2000.

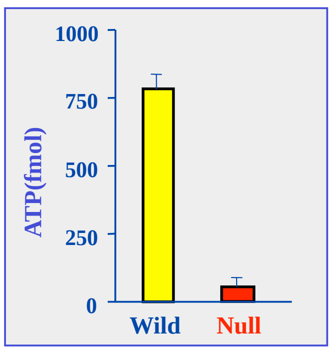
Distension evoked ATP release in bladders from TRPV1 null mice was done to evaluate whether abnormal bladder function in TRPV1 null mice *may be due to changes in neuronal detection of bladder stretch*

Bladder Stretch (in both cells and tissue) evokes ATP Release

Bladders mounted in Ussing chambers ; hydrostatic pressure applied to the mucosal surface



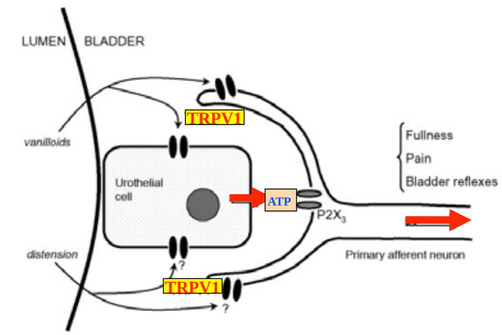




TRPV1 gene disruption alters purinergic signaling in the urothelium---by impairing the ability of urothelial cells to release ATP in response to stretch

Involvement of TRPV1 and the urothelium in signaling of sensory events

- ***TRPV1-urothelial receptors are activated by vanilloids (as well as distension) causing ATP release, activating P2X$_3$ receptors on afferents***
- ***This can lead to sensory events---such as fullness or pain, and activation of bladder reflexes***

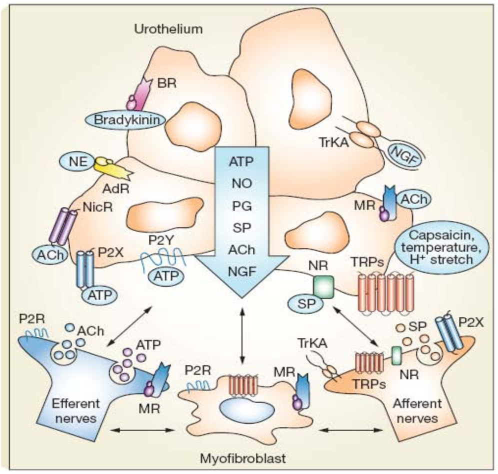


In neurons, TRPV1 is thought to integrate/amplify the response to various stimuli (plays essential role in development of inflammation-induced hyperalgesia)

(Recent studies also demonstrate a role for TRPV1 in excitability of low threshold bladder afferents---Daly et al.,J Physiol 583:663-74, 2007)

Urothelial – TRPV1 might participate in a similar manner, in the detection of irritant stimuli following bladder inflammation/infection

Urothelial cells can receive and integrate multiple stimuli---providing an important "link" in the transfer of information from the urinary bladder to the nervous system



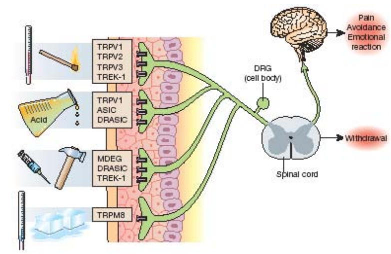
Birder and de Groat; Nat Clin Prac Urol 4:46-54, 2007

The release of transmitters/mediators may form the basis of a "chemical-cross talk" within a number of cell-types in the bladder wall

Urothelial cells are likely to play a role in pain processes/sensory function

Recent evidence has demonstrated that urothelial cells exhibit plasticity

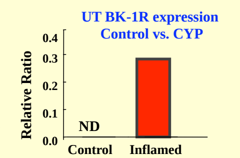
Inflammation/injury can alter the "sensor function" ***(expression & sensitivity)*** **of urothelial targets which could contribute to hypersensitivity and pain**



Urothelial expression of bradykinin receptors are plastic and can be altered by pathology

CYP-inflammation increases UT-BK-1 receptor expression

- Correlates with increased bladder hyperactivity
- BK-receptor antagonists (intravesically) reduces hyperactivity



Chopra et al., J Physiology 562:859-71, 2005.

This can lead to changes in "urothelial transducer function" (release of chemicals or neurotransmitters), which can impact on both urothelial function and neural excitability

Urothelial Cells Can Synthesize/Release a Number of Mediators:

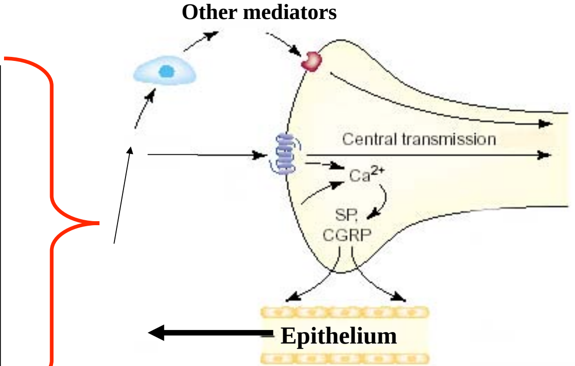
ATP

Prostaglandins

Nitric Oxide

Acetylcholine

Nerve Growth Factor



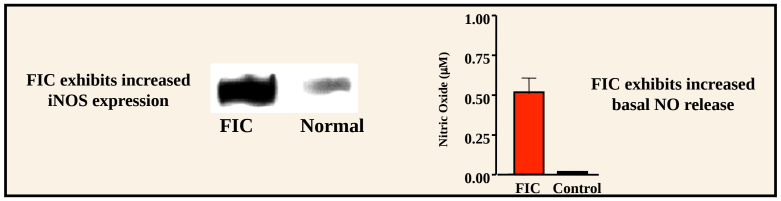
Altered Urothelial Receptors and Substances in Bladder Dysfunction:

Painful Bladder Syndrome / Interstitial Cystitis

Nitric Oxide signaling mechanisms are altered in PBS/IC

- Cats diagnosed with PBS/FIC exhibit an increased iNOS expression & increased basal NO release; due to activation of iNOS

Birder et al., J Urol 173: 625-9, 2005.

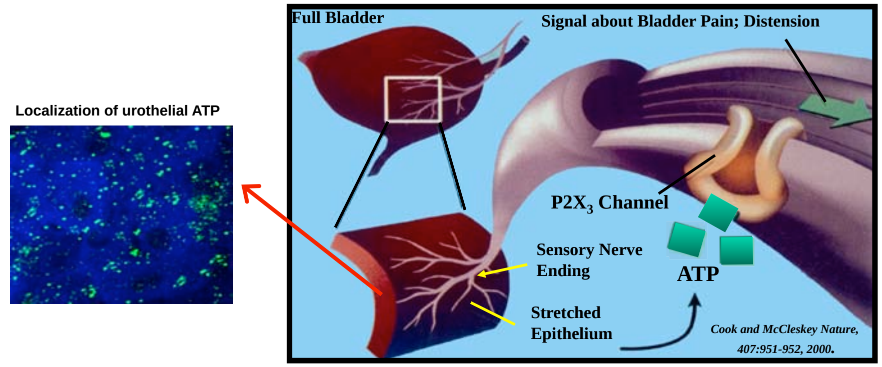


- Patients diagnosed with PBS/IC exhibit increased iNOS expression (mainly in urothelium); increased NO in urine of patients with "classic" IC as compared to non-ulcer IC.

Logadottir et al., J Urol, 171: 1148-50. 2004; Koskela et al J Urol 2008

The role of elevated NO in PBS/IC still remains to be determined

During distension, ATP release from urothelium—via activation of suburothelial afferents--may trigger sensations of fullness, pain or changes in bladder activity



Large amounts of ATP released from urothelial cells in FIC may alter afferent excitability (via $P2X_3$ receptors on pelvic afferents); triggering the sensation of pain

Summary:

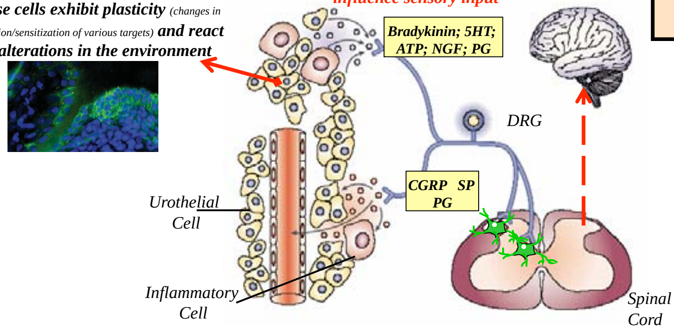
Pathology can lead to changes in both "sensor" and "transducer" properties of urothelial cells

Urothelial cells responds to physical and chemical stimuli via release of a number of signaling molecules.

These cells exhibit plasticity (changes in expression/sensitization of various targets) and react to alterations in the environment

Increased release of neurotransmitters/inflammatory mediators (from urothelial, inflammatory and/or neural cells)-can influence sensory input

Alterations in the "response" of non-neuronal cells may ultimately influence bladder function



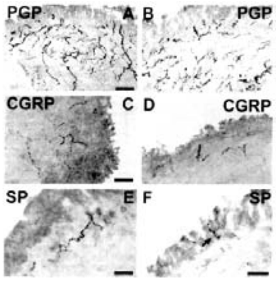
Bladder sensory desensitization decreases urinary urgency

(TRPV1 receptors and bladder sensation)

Francisco Cruz

Hospital S João / Faculty Medicine of Porto
Portugal

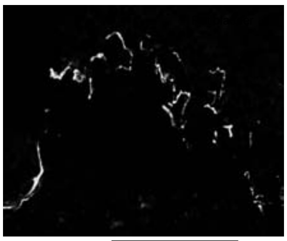
SP- and CGRP-IR fibre density in the bladder of IDO patients



Smet et al, 97

Co-localization of TRPV1 with SP or CGRP

TRPV1
CGRP
TRPV1+CGRP



TRPV1
SP
TRPV1+SP

Avelino et al, Neuroscience, 2002

TRPV1-IR fibre density in the bladder of IDO patients

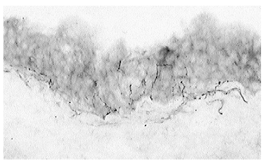


TABLE 3. *Changes in values of suburothelial PGP9.5-IR, $P2X_3$-IR and TRPV1-IR fibers after treatment with intradetrusor BoNT/A*

	Mean ± SEM		
	PGP9.5	$P2X_3$	TRPV1
Controls		0.47 ± 0.09	0.21 ± 0.07
DO:			
Baseline	2.00 ± 0.29	1.39 ± 0.13	0.39 ± 0.08
4 Wks	1.95 ± 0.25	0.85 ± 0.13	0.28 ± 0.05
16 Wks	1.72 ± 0.29	0.49 ± 0.12	0.12 ± 0.04
p Value (pretreatment vs 4 wk, No.)	0.85, 21	0.009, 24	0.19, 22
p Value (pretreatment vs 16 wk, No.)	0.21, 13	0.0004, 15	0.0008, 16
NDO:			
Baseline	2.36 ± 0.33	1.44 ± 0.15	0.42 ± 0.09
4 Wks	1.97 ± 0.31	0.89 ± 0.15	0.32 ± 0.07
16 Wks	1.72 ± 0.31	0.46 ± 0.14	0.10 ± 0.03
p Value (pretreatment vs 4 wk, No.)	0.14, 15	0.02, 18	0.32, 16
p Value (pretreatment vs 16 wk, No.)	0.16, 12	0.001, 13	0.0008, 14
IDO:			
Baseline	1.49 ± 0.28	1.24 ± 0.25	0.33 ± 0.14
4 Wks	1.64 ± 0.28	0.73 ± 0.21	0.19 ± 0.03
p Value (pretreatment vs 4 wk, No.)	0.74, 6	0.13, 6	0.43, 6

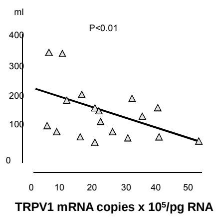
Baseline values refer to patients with available followup biopsies.

Apostolidis et al, *J Urol.*, 2005

TRPV1 expression in patients with urgency

TRPV1 mRNA correlates inversely with bladder volume to 1st bladder sensation in patients with overactive bladder

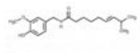
Volume to first bladder sensation to urinate



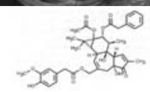
Liu et al, Neurourol Urodyn, 26:433, 2007

TRPV1 desensitization

Capsaicin



Resiniferatoxin



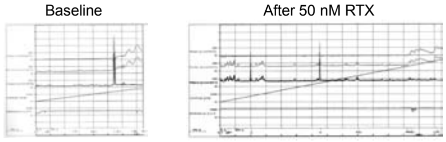
Resiniferatoxin is much less pungent than capsaicin instillation but equally effective in desensitizing TRPV1

Cruz et al, Lancet, 1997

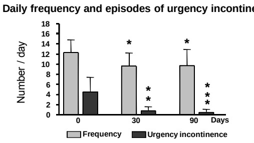
TRPV1 desensitization improves patients with Idiopathic Detrusor Overactivity

Baseline

After 50 nM RTX



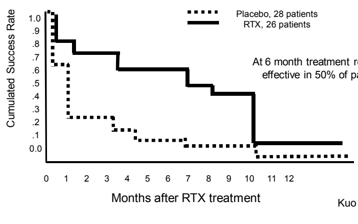
Daily frequency and episodes of urgency incontinence



Silva, Ribeiro and Cruz, *J Urology*, 2002

Intravesical RTX in IDO patients

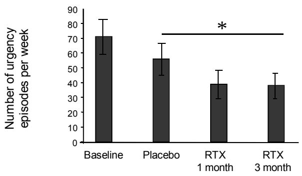
RTX instillations in patients with refractory IDO



Kuo et al, J. Urol 2006

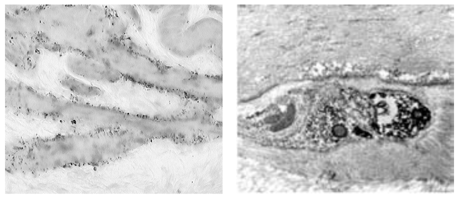
Effect of intravesical RTX on urgency

0: No bladder sensation
1: Micturition with a normal sensation
2: Urge but with full control of micturition
3: I had to abandon what I was doing
4: I had to abandon what I was doing and I leaked before reaching the toilette



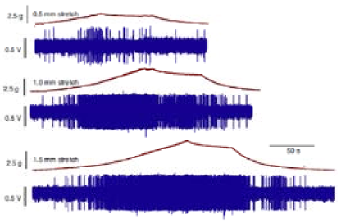
Silva, et al, BMC Urology, 2007

Relationship between smooth muscle cells and sensory fibers (TRPV1-IR)



Avelino et al, *Neuroscience*, 02

Changes in bladder tension and firing of single afferent fiber

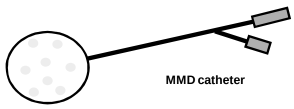


Localized contractions may evoke afferent activity

McCarthy et al, *J Urol*, 2009

Micromotions/segmentar contractions

Occurrence of localized bladder contractions



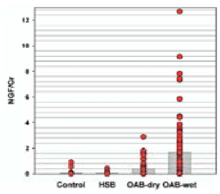
Normal volunteers 2/6 (30%)

Patients with urgency...12/14 (85%)

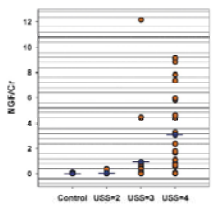
P=0.02

Drake et al, *BJU Int*, 2005

NGF in the urine of OAB patients



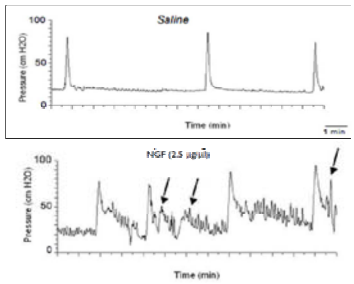
Liu HT et al, *J Urol*, 2008



Liu et al, Eur Urol, 2008

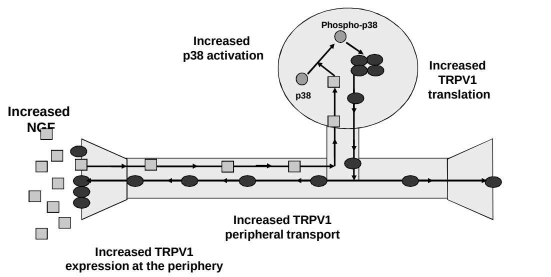
NGF/Cr: Nerve growth factor/creatinine
HSB: hypersensitive bladder
OAB: overactive bladder
USS: Indevus urgency severity scale (0-4)

Effect of exogenous NGF on rat bladder



Zvara & Vizzard, *BMC Physiology*, 2007

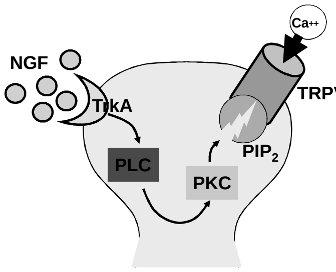
NGF regulates TRPV1 during inflammation



NGF
TRPV1

Adapted from Ji et al, *Neuron*, 2002

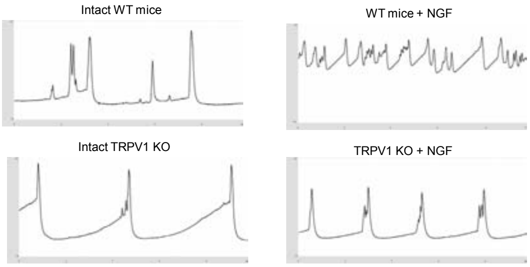
NGF increases TRPV1 activity



PLC: phospholipase C
PKC: protein kinase C
PIP_2: phosphatidylinositol-4,5-bisphosphate

Adapted from:
Chuang et al, *Nature*, 2001
Szallasi and Fowler, *Lancet Neurol.* 2002

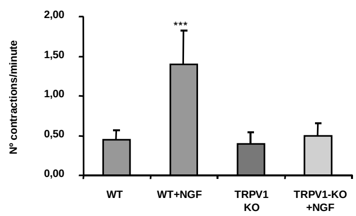
Importance of TRPV1 for the NGF effect on bladder function



NGF: 100 µg/kg weight IP x 3days

Charrua et al, Neurourol Urodyn 2009 (abstract)

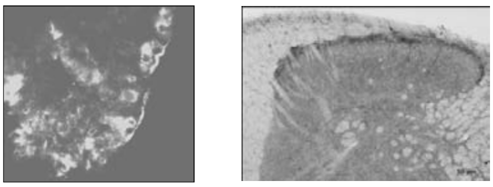
Importance of TRPV1 for the NGF effect on bladder function



NGF: 100 µg/kg weight IP x 3days

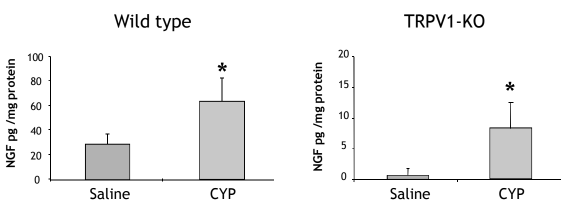
Charrua et al, Neurourol Urodyn 2009 (abstract)

TrkA immunoreactivity in TRPV1-KO mice



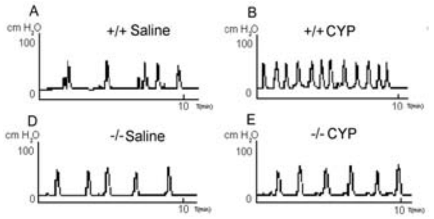
Charrua et al, Neurourol Urodyn 2009 (abstract)

Bladder NGF after CYP- induced cystitis



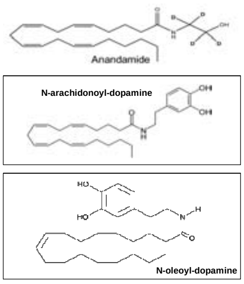
Charrua et al, Neurourol Urodyn 2009 (abstract)

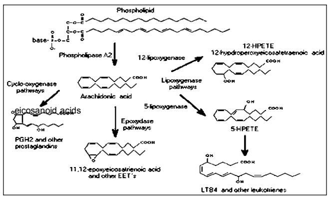
TRPV1 is essential for the development of detrusor overactivity caused by cystitis.



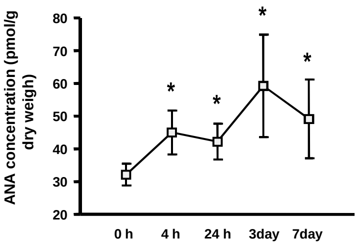
Charrua et al, J Urol, 2007

Endogenous TRPV1 agonists



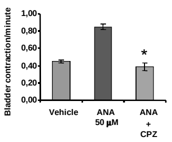


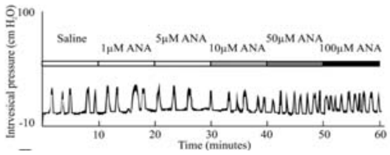
Anandamide concentration in normal and CYP-inflamed bladders

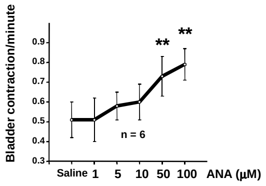


Dinis et al, J Neurosci, 2004

Effect of anandamide on bladder function

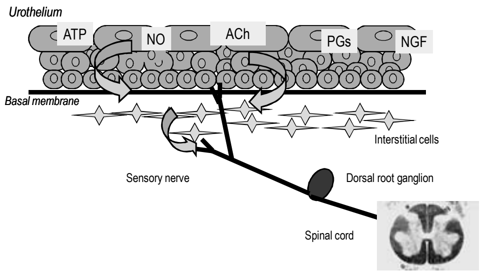






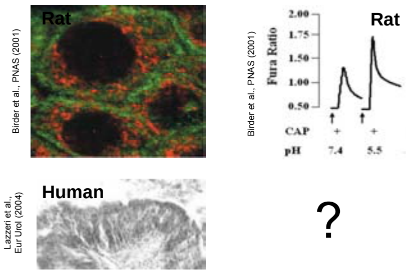
Dinis et al, J Neurosci, 2004

BoNT-A may interfere with urothelium-suburothelium nerve fiber cross-talk

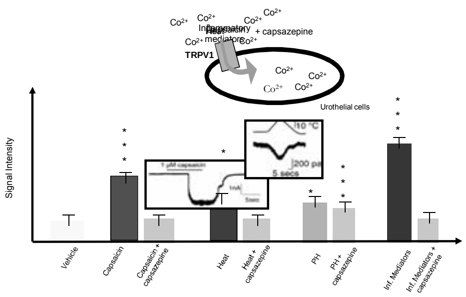


K-E Andersson

Is TRPV1 expressed (and functional) in human urothelium?



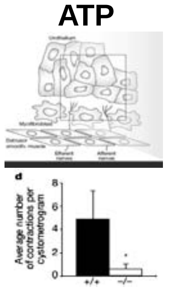
Functional proprieties of human urothelial TRPV1



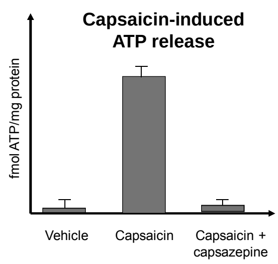
Charrua et al., J. Urol (2009b)

TRPV1-ATP interaction in human urothelium

ATP

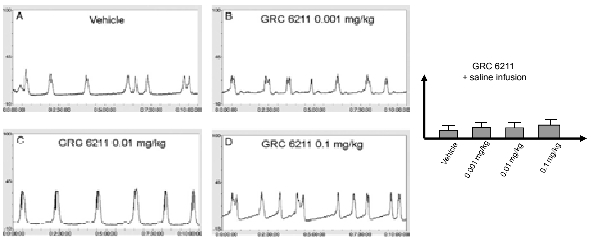


Cockayne et al., Nature, 00



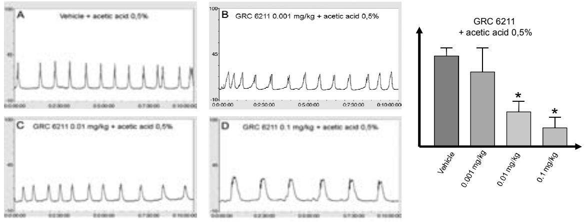
Charrua et al., J. Urol, 2009

Effect of a TRPV1 blockade on bladder reflex activity of intact animals



Charrua et al., J. Urol (2009)

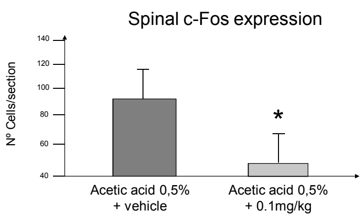
Effect of TRPV1 blockade on reflex activity of acute inflamed bladders



TRPV1 antagonist: GRC-6211

Charrua et al., J. Urol (2009)

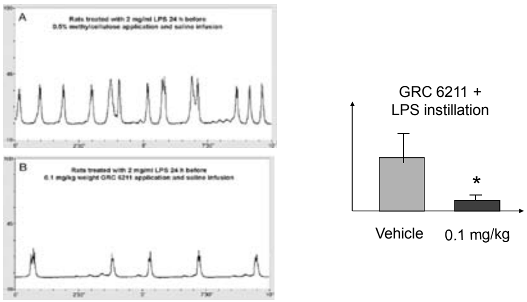
Effect of TRPV1 blockade on sensory input generated by acute inflamed bladders



TRPV1 antagonist: GRC-6211

Charrua et al., J. Urol (2009)

Effect of TRPV1 blockade on reflex activity of chronic inflamed bladders



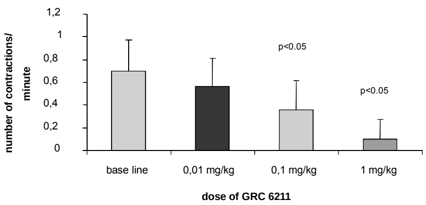
TRPV1 antagonist: GRC-6211

Charrua et al., J. Urol (2009)

Effect of cumulative doses of TRPV1 antagonist on Detrusor Overactivity of SCI rats

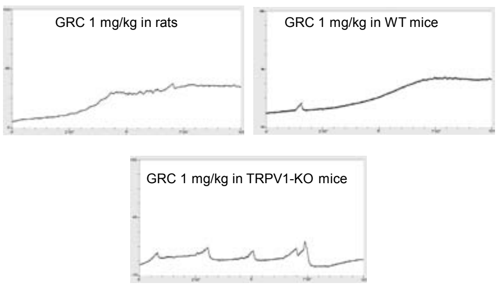
Silva, A et al, Neurourol Urodyn, 2008

Effect of cumulative doses of TRPV1 antagonist on Detrusor Overactivity of SCI rats



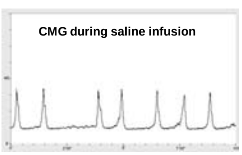
Silva, A et al, Neurourol Urodyn, 2008

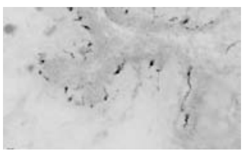
Effect of high doses (1 mg/kg) of GRC 6211 on bladder function of intact rats and WT and TRPV1-KO mice



Charrua *et al*, J Urol, 2009

Bladder function and TRPV1-IR 24 hours after administration of 1 mg/kg of GRC 6211 to intact rats

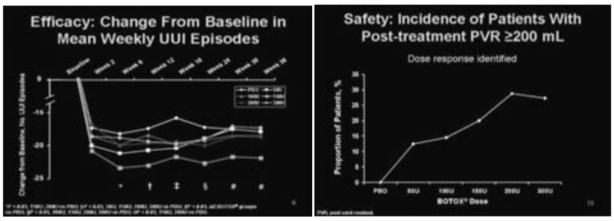




TRPV1-IR

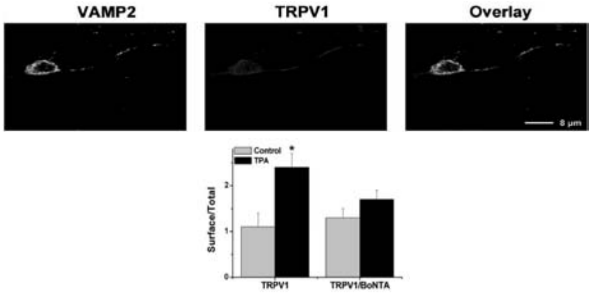
Charrua *et al*, J Urol, 2009

First large, double-blind, placebo-controlled study to assess the benefit/risk of BOTOX® in idiopathic OAB



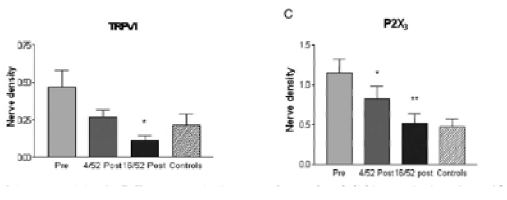
Chapple et al, Eur Urol Suppl, 2010, (Abstract)

BoNT-A prevents SNARE-dependent TRPV1 trafficking in sensory cells



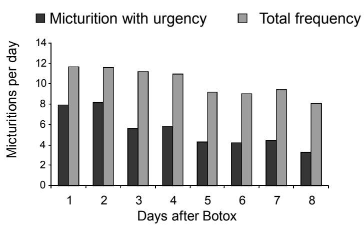
Morenilla-Palao et al, *J Biol Chemistry*, 2004

BoNT-A decreases TRPV1 and $P2X_3$ immunoreactivity in the suburothelium of NDO patients



Apostolidis et al, *J Urol.*, 2005

Decline of urgency after 200 U of Botox in patients with NDO (n=17) and IDO (n=11)



Adapted from
Kalsi et al, Eur Urol 2008

Conclusions

- **TRPV1 seems essential for bladder activity in IDO, NDO or inflammation**
- **TRPV1 expression is essential for NGF-induced bladder overactivity**
- **Endogenous ligands of TRPV1 should be evaluated in bladder dysfunction**
- **RTX or BoNT/A can be used to counteract TRPV1**
- **TRPV1 antagonists might be relevant therapeutic tools for visceral pain**

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Istvan Nagy

Neuropilins are at the cross-road of the vascular and nerve system

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http://sabanlab.org

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Views and opinions of, and endorsements by the author do not reflect those of the US Army or the Department of Defense.

1

Neuropilins are at the cross-road of the vascular and nerve system

1 Vascular and Nerve Systems

Just recently, the common molecular basis of neuron and vessel specification, growth, navigation, and survival has started to become elucidated.

The vascular endothelium growth factor (VEGF) and its receptors play a key role in angiogenesis and neurogenesis. *Physiol. Rev. 89: 607–648, 2009.*

2 Neuropilins

Neuropilins (NRP1 and NRP2) are non-tyrosine kinase transmembrane proteins that are crucial for the embryonic development of neural and vascular systems.

3 NRPs as Co-receptors

NRP1 and NRP2 are cue molecules that guide both axonal and vascular growth.

NRPs along with Plexin regulate neuronal and axonal patterning.

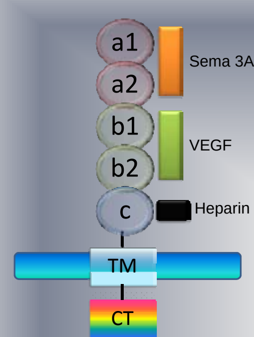
NRPs along with VEGFRs regulate vessel navigation.

2

NEUROPILINS

- Non-tyrosine kinase proteins
- Co-receptor for plexin – semaphorin – axon guidance
- New co-receptors for VEGF
- Involved in angiogenesis, lymphangiogenesis, and immune responses
- Expressed also outside the vascular system – renal epithelial cells
- Roles: cancer and inflammation

3

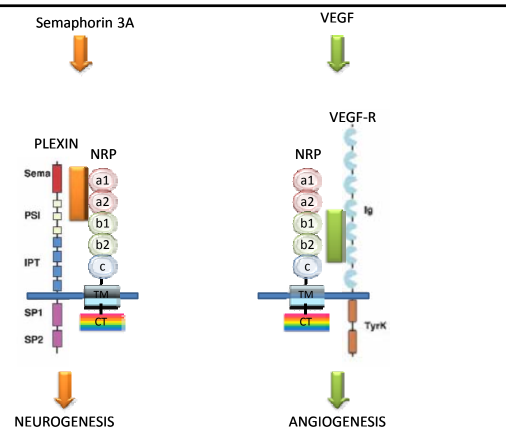


A substantial fraction of NRP is proteoglycan modified with either heparan sulfate (HS) or chondroitin sulfate (CS)

Exogenous heparin and HS bind NRP and increase VEGF binding to NRP1 and VEGFR2

The EMBO Journal (2006) 25, 3045–3055

4



Genes & Development 19:1013, 2005

5

Other NRPs Ligands

- Semaphorins
- Heparin-binding proteins
- Fibroblast growth factor 2
- Hepatocyte growth factor
- Placental growth factor 2
- Synthetic peptides (Tuftsin)

6

Using scVEGF-Cy5.5 to access active VEGF receptors

1. scVEGF-Cy5.5 contains two fused fragments of $VEGF_{121.}$
2. scVEGF-Cy5.5 is freely distributed because $VEGF_{121}$ lacks the AA residues responsible for heparin binding.
3. scVEGF-Cy5.5 is internalizable.
4. scVEGF-Cy5.5 activity is comparable to native $VEGF_{121}$:

   4a. Induction of VEGFR-2 tyrosine auto-phosphorylation,
   4b. Competition with a VEGF-toxin for binding to VEGFRs
   4c. Induction of VEGFR-mediated internalization.

Unlike IF that shows all cells expressing VEGFRs, scVEGF/Cy5.5 tracer identifies only cells with **accessible** and **functionally active** VEGFRs.

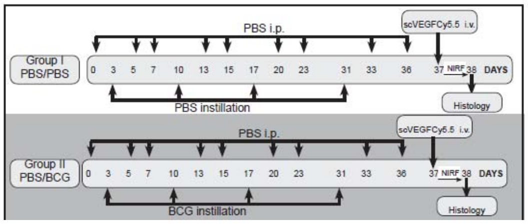
7

BCG-induced inflammation increases urothelial expression of VEGFRs and NRPs

1 Methods	2 Results	3 Conclusion
We determined the nature of cells responding to VEGF in normal and inflamed bladders by tagging such cells *in vivo* with a targeted fluorescent tracer, scVEGF/Cy5.5 which identifies cells with accessible and **functionally active** VEGF receptors.	Inflammation was induced by intravesical instillation of BCG (Bacillus Calmette-Guerin). *In vivo* NIRF imaging with i.v. injected scVEGF/Cy5.5 revealed accumulation of Cy5.5 in both urothelial and ganglial cells, expressing VEGF receptors VEGFR-1 and VEGFR-2, as well as NRP1 and NRP2.	Inflammation induced up-regulation of VEGFRs and NRPs genes and protein expression. Our results strongly suggest new and blossoming VEGF-driven processes in bladder urothelial cells and ganglia in the course of inflammation.

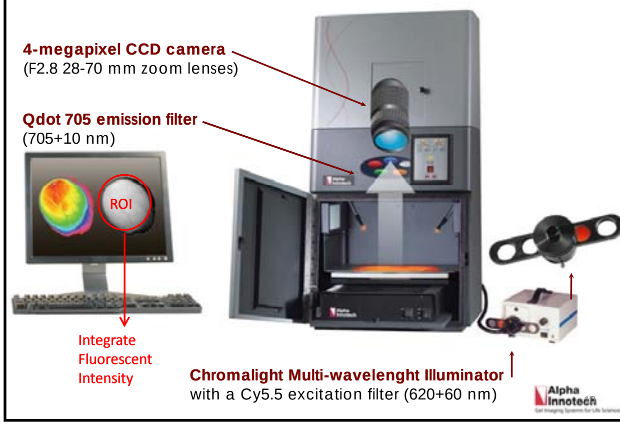
AJP-Renal 295:F60, 2008

8

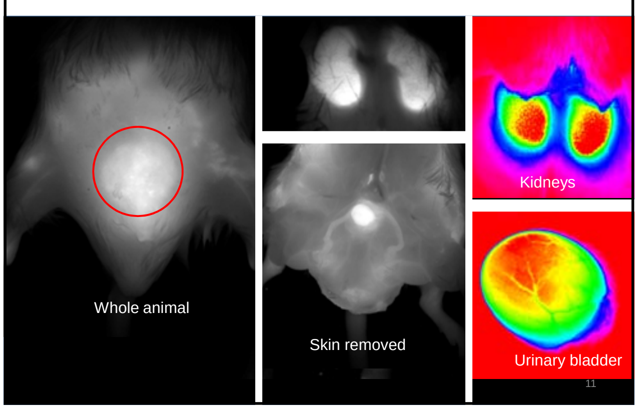


9

FluorChem® HD2 Cabinet



Intravenous administration of scVEGF-Cy5.5 leads to a time-dependent accumulation of fluorescence in the urinary bladder and kidneys.



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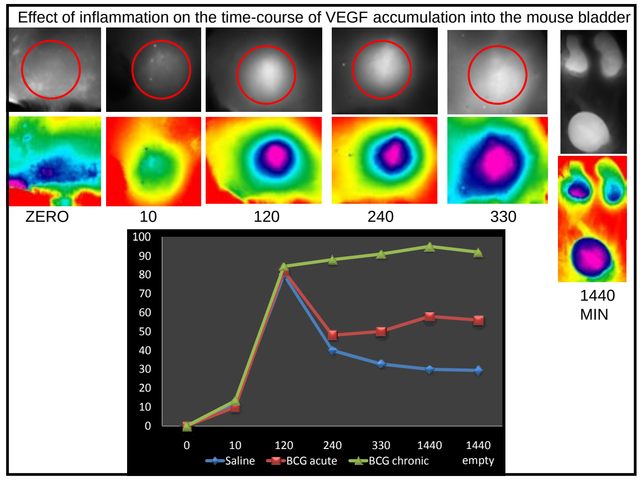
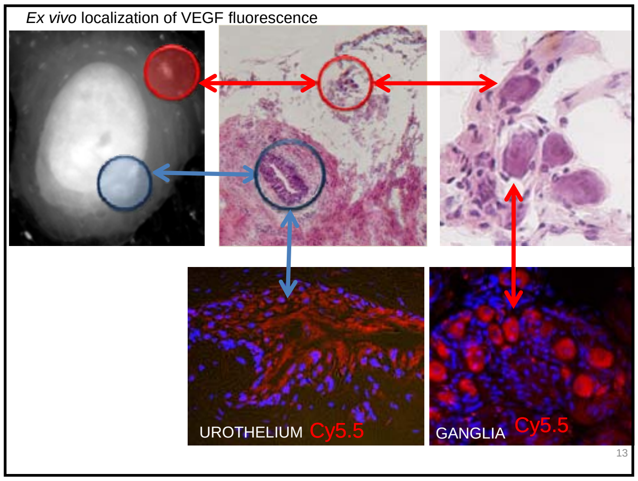
Effect of inflammation on the time-course of VEGF accumulation into the mouse bladder





Ex vivo localization of VEGF fluorescence
UROTHELIUM Cy5.5
GANGLIA Cy5.5
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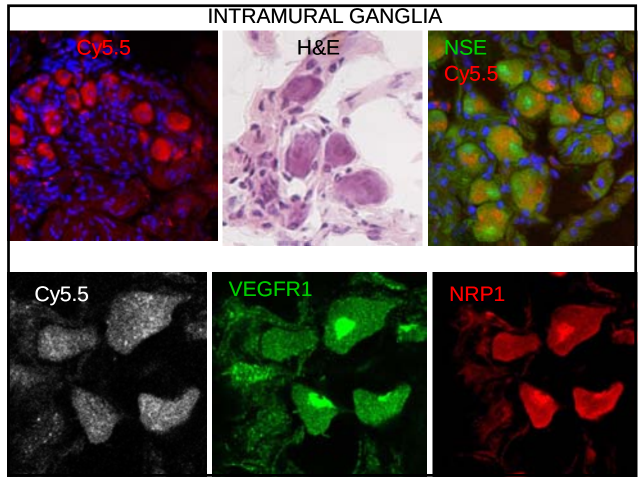
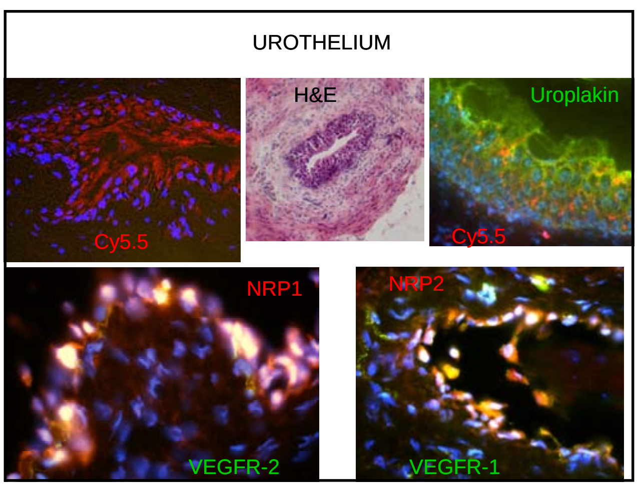
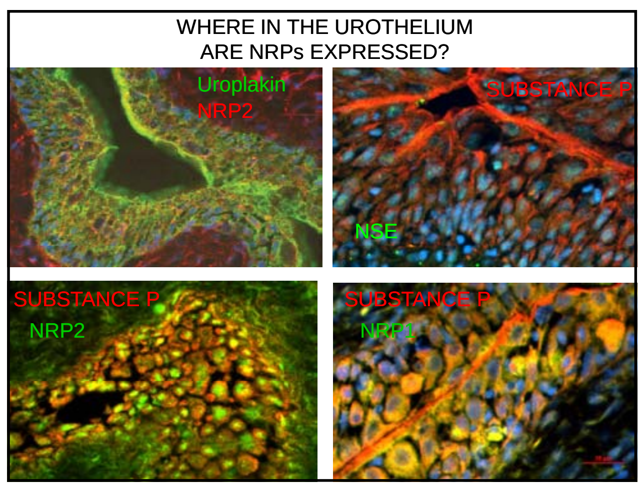


INTRAMURAL GANGLIA
Cy5.5
H&E
NSE
Cy5.5
Cy5.5
VEGFR1
NRP1

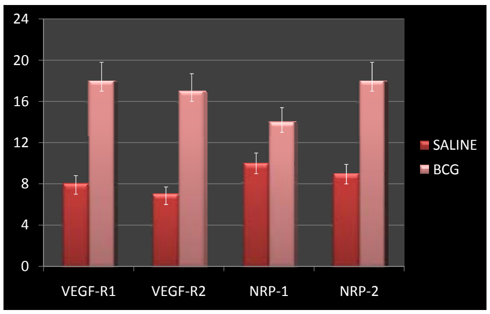


UROTHELIUM
Cy5.5
H&E
Uroplakin
Cy5.5
NRP1
VEGFR-2
NRP2
VEGFR-1



WHERE IN THE UROTHELIUM
ARE NRPs EXPRESSED?
Uroplakin
NRP2
SUBSTANCE P
NSE
SUBSTANCE P
NRP2
SUBSTANCE P
NRP1

BCG induces increased VEGFRs and NRPs protein expression



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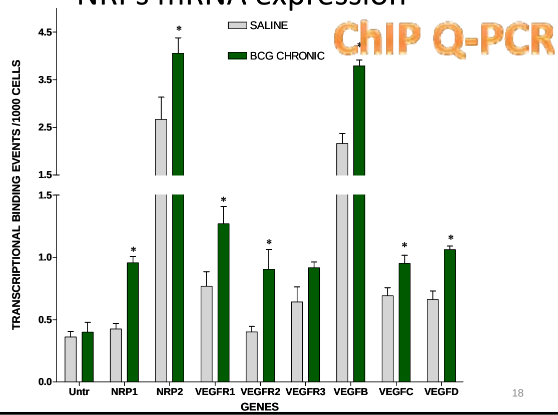
BCG induces increased VEGFRs and NRPs mRNA expression



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VEGFRs and NRPs are expressed in human urothelium and decreased in IC

We reported that VEGFs and NRPs are strongly expressed in both: a) human bladder urothelium , and b) human bladder cancer cell line (J82).
In addition, the expression of NRP2 and VEGFR1 were significantly down-regulated in IC bladder biopsies when compared to control subjects.

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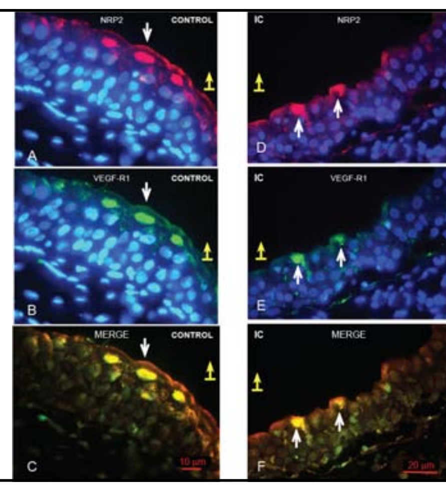

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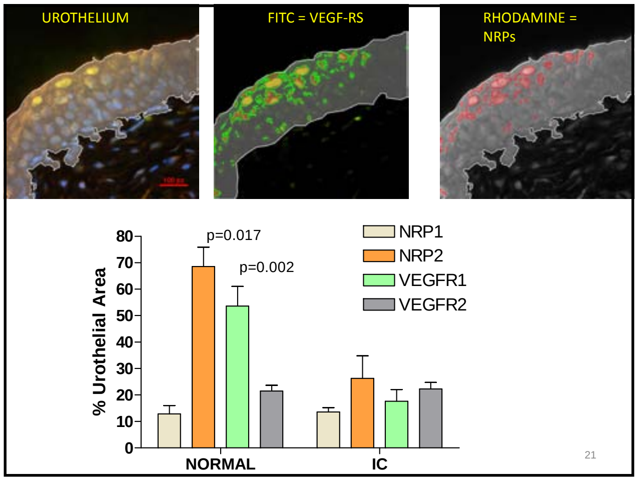

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VEGF-neuropilin signaling pathway acts as a key modulator of vascular, lymphatic and inflammatory cell responses of the bladder to intravesical BCG treatment.

1 Methods	2 Results	3 Conclusion
We investigated the role of VEGFRs and NRPs in the mouse bladder responses to intravesical BCG. Mice received intraperitoneal injections of PBS, anti-NRP1^A or anti-NRP1^B neutralizing antibodies, and then were challenged chronically with intravesical PBS or BCG. At the end of chronic challenge period, the uptake of scVEGF/Cy5.5 was determined in vivo and real time by NIRF.	BCG increased the density of blood and lymphatic vessels. Treatment of the mice with NRP neutralizing antibodies dramatically reduced scVEGF/Cy5.5 uptake, PMNs and CD11c infiltration, blood and lymphatic vessels density.	These results implicate NRPs as critical *in vivo* regulators of the vascular and inflammatory responses to the intravesical administration of BCG.

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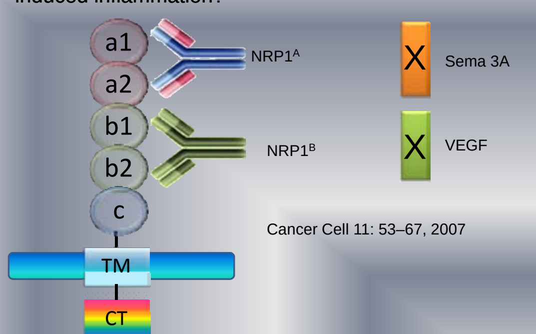
Do anti-NRP neutralizing antibodies decrease BCG-induced inflammation?



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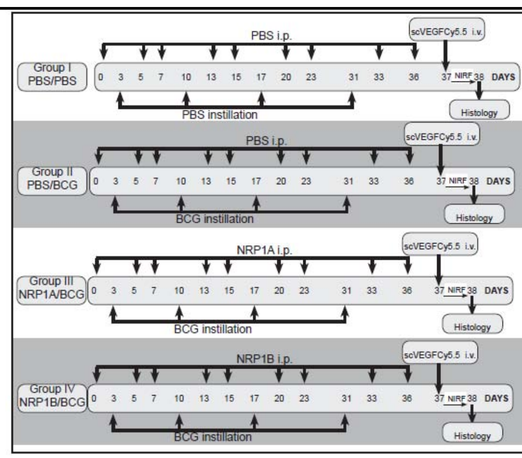

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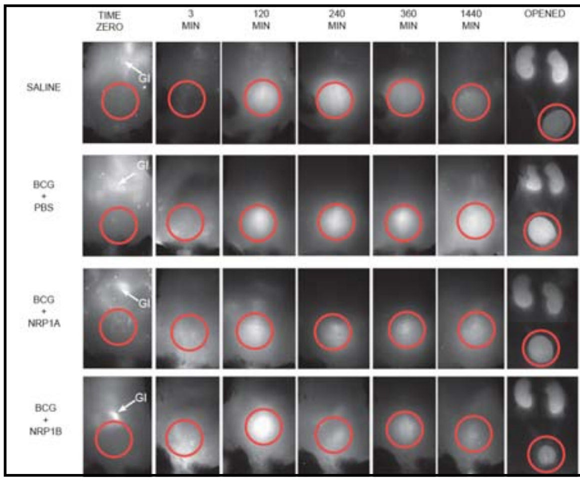

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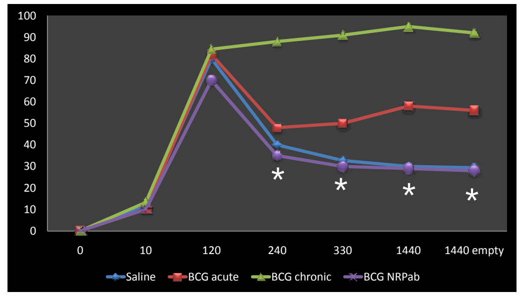
Anti-NRP antibodies alter the accumulation of scVEGF/Cy5.5



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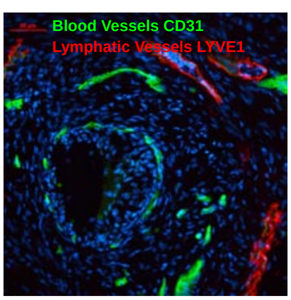
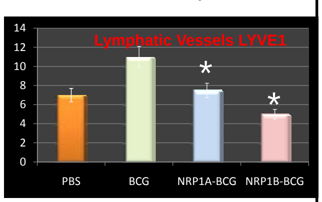
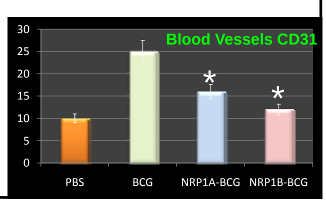
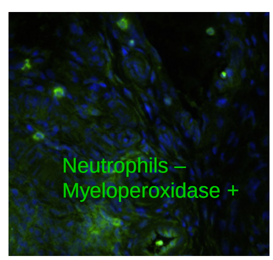
Anti-NRP abs block BCG-induced increase of blood and lymphatic vessels density

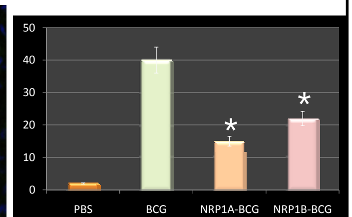






Anti NRP abs block BCG-induced neutrophil infiltration





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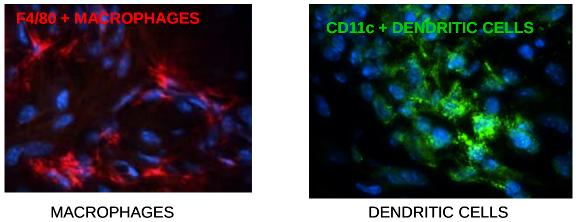
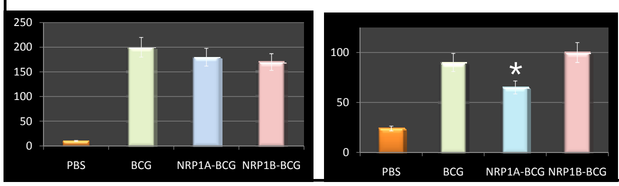
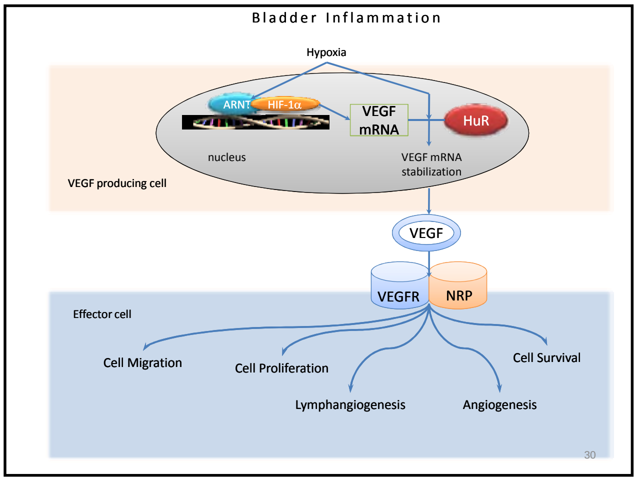
Anti NRP1^A ab blocks BCG-induced dendritic cell infiltration







Conclusions

- scVEGF/Cy5.5 is a powerful tool for determination of active VEGF receptors.
- Our studies with scVEGF/Cy5.5 resulted in the discovery of NRPs and VEGFRs in the bladder urothelium and intramural ganglia.
- In the urothelium, NRPs are co-localized with SP-containing nerves.
- Anti-NRP antibodies reduced blood vessel density, lymphatic vessel density, and inflammatory cell migration.
- NRPs and VEGFRs seems to mediate the inflammatory arm of BCG therapy.

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