#603 Complement activation mechanism by autoantigen recognition in growth process of benign prostatic hyperplasia

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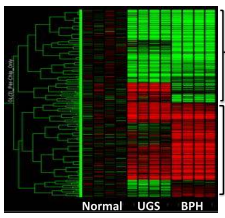
【Background】

Microarray analysis*



down-regulation

up-regulation

Functional network analyses

up-regulation
down-regulation

inflammation response pathway: Thrombospondin 1, Col1a1, Col1a2, Col3a1,Mmp2,Lamb1,Lamb2, Lamc1, fibronectin 1, Il2rg,

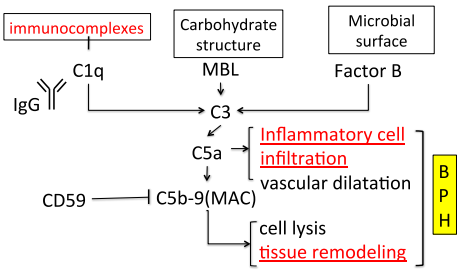
Complement classical pathway: C1q, C1r, C2, Tfpi, F2r, Plat Cd59(regulatory factor), Crry, Pros1

(Hata J, et al; Int J Urol 2016)

* Affimetrix system (Santa Clara, CA, USA)
Gene Spring® 7.3.1 (Agilent Technologies, Santa Clara, CA, USA)

*BPH; Benign Prostatic Hyperplasia

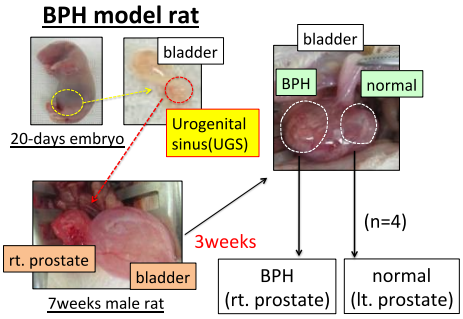
Complement activation pathway



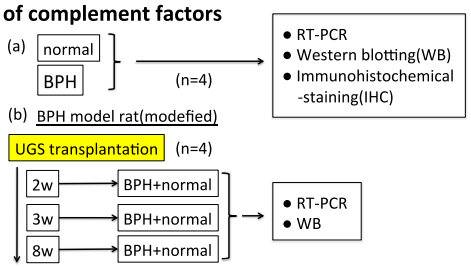
The association between the pathogenesis of benign prostatic hyperplasia (BPH) and inflammation has recently received attention. We previously showed that both the inflammation response pathway and the classical complement pathway are activated in BPH tissue from model rats with stroma-dominant BPH. The classical complement pathway is activated by autoantigens that recognize immunocomplexes and is responsible for various diseases via a mechanism that amplifies inflammation. We postulated that immunocomplexes amplify inflammation through complement activation, which leads to prostatic proliferation. Therefore, we expressed complement factors, analyzed their functions, and identified autoantigens to understand the pathogenic mechanism of BPH.

【Material and Methods】

BPH model rat



Study 1. Expression and functional analysis of complement factors

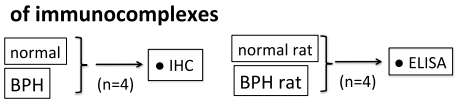


Study 2. Expression of complement factors in human BPH

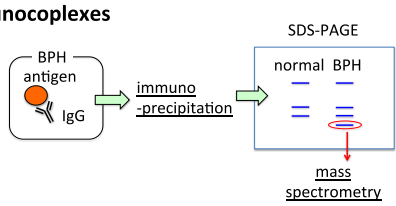
- human BPH (n=4)(prostate biopsy, TUR-P)
- human normal prostate (n=4)(total cystectomy with no cancer)

● IHC

Study 3. Expression and functional analysis of immunocomplexes

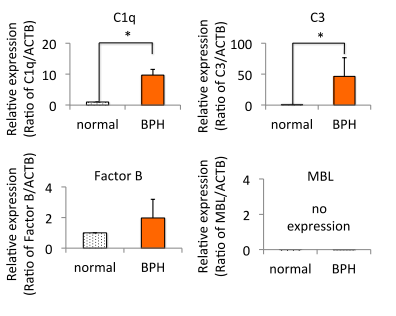


Study 4. Identification of autoantigen binding Immunocomplexes

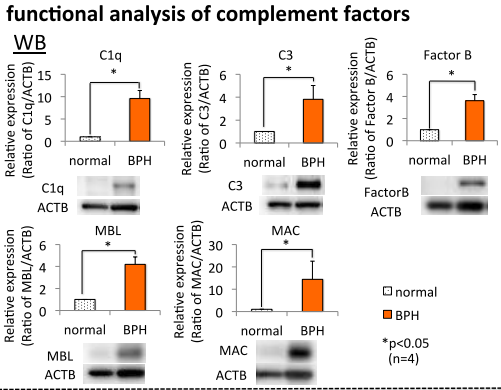


【Results】 Study 1. Expression and functional analysis of complement factors

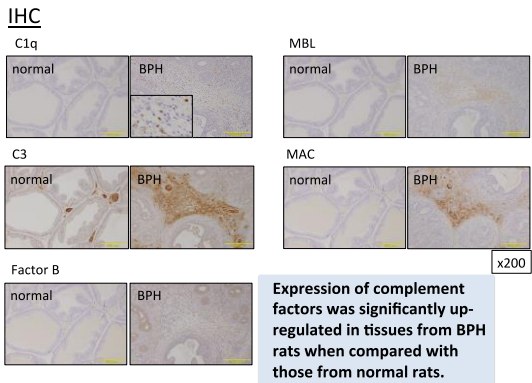
(a) RT-PCR



WB

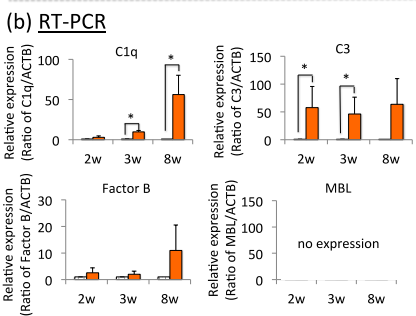


IHC

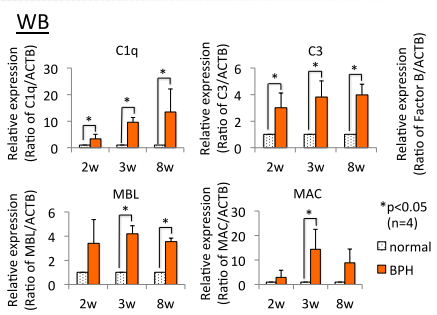


Expression of complement factors was significantly up-regulated in tissues from BPH rats when compared with those from normal rats.

(b) RT-PCR

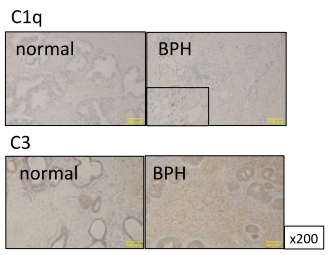


WB



The classical complement pathway was initially activated, followed by an alternative complement pathway activated in BPH.

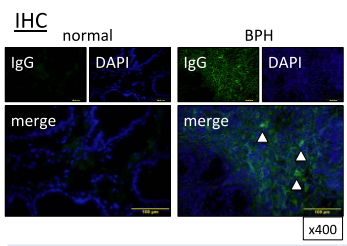
Study 2. Expression of complement factors in human BPH



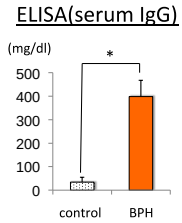
These complement factors were also up-regulated mostly in stromal areas of human BPH.

Study 3. Expression and functional analysis of immunocomplexes

IHC



ELISA(serum IgG)

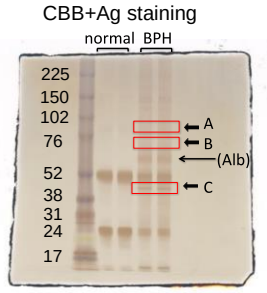


Serum IgG concentration was significantly increased (398.1 ng/mL, p<0.01) in rat BPH and IgG was deposited in stromal areas of the BPH.

Study 4. Identification of autoantigen binding Immunocoplexes

Immunoprecipitation

CBB+Ag staining



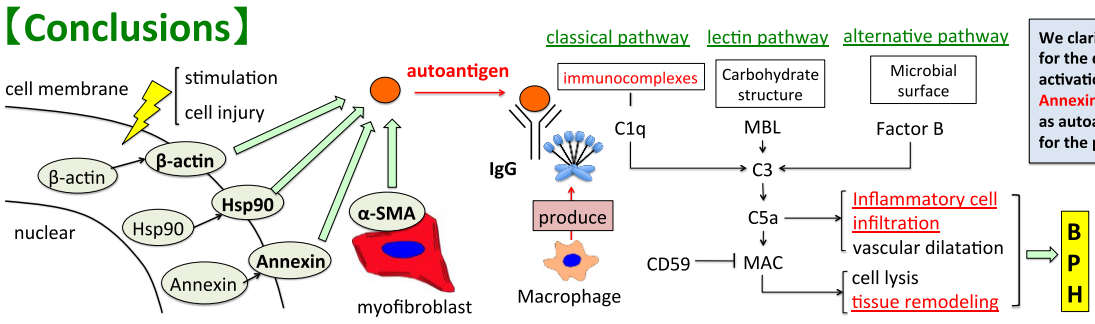
mass spectrometry

lane	Score	protein	Mw (Da)
A	965	Heat shock protein HSP 90-beta	83,229
	617	Heat shock protein HSP 90-alpha	84,762
B	*1	Annexin	75,706
C	2225	Actin, cytoplasmic 1(β-actin)	41,710
	1420	Actin, aortic smooth muscle (α-SMA)	41,982

- Database：Uniprot-SwissProt
- Server：Mascot□erver （MATRIX□CIENCE）/ Sequest HT (Thermo Fisher Scientific Inc.)

Mass spectrometry of IgG binding protein identified Annexin, Hsp90, α-SMA and β-actin as antigens of immunocomplexes.

【Conclusions】



We clarified that the immune system is responsible for the development of BPH. Complement pathway activation by immunocomplexes recognizing Annexin, Hsp90, α-SMA and β-actin as autoantigens might be responsible for the pathogenesis of BPH.