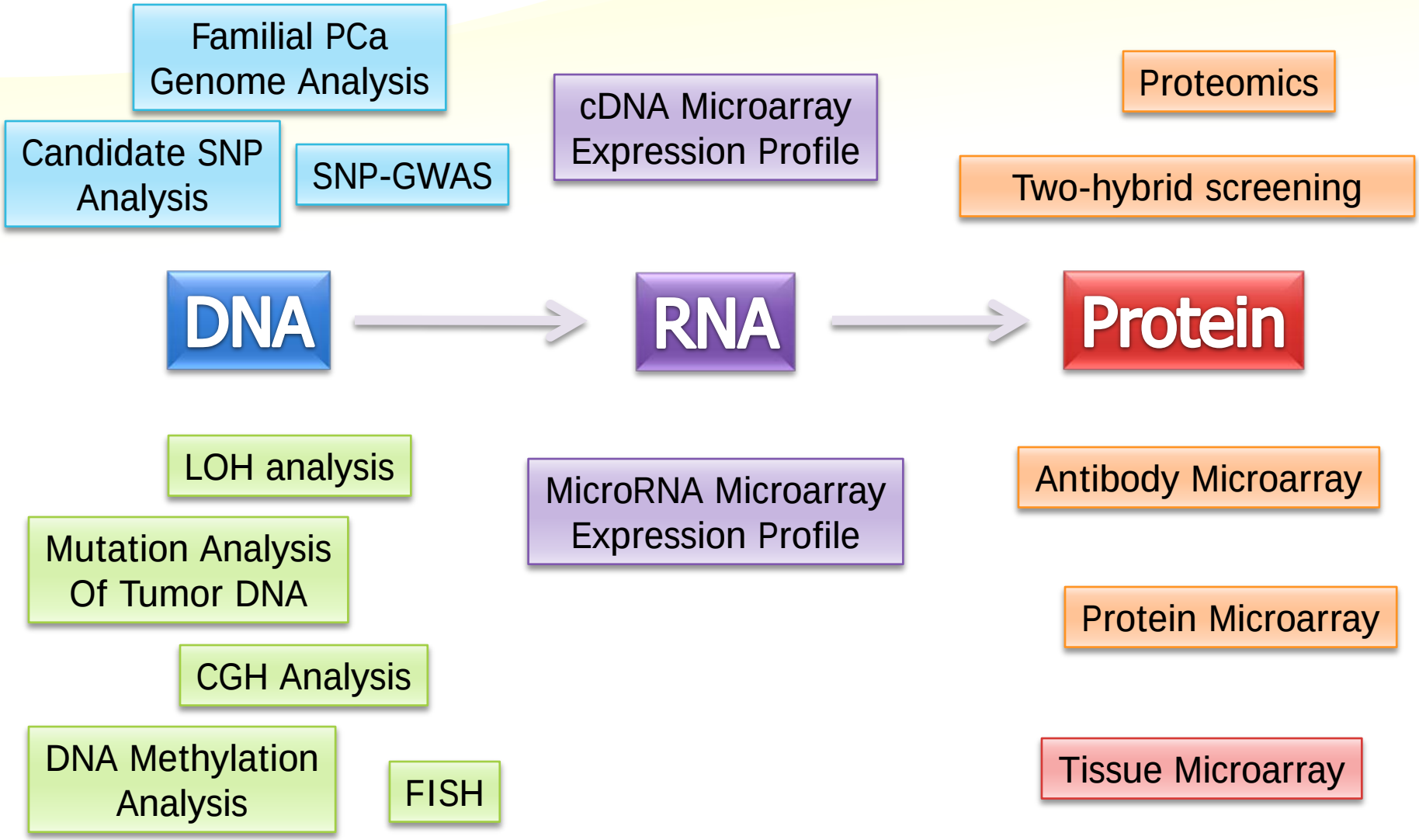
Genomics & Epigenomics in Prostate Cancer:

Toward the Identification of New Candidate Molecular Targets

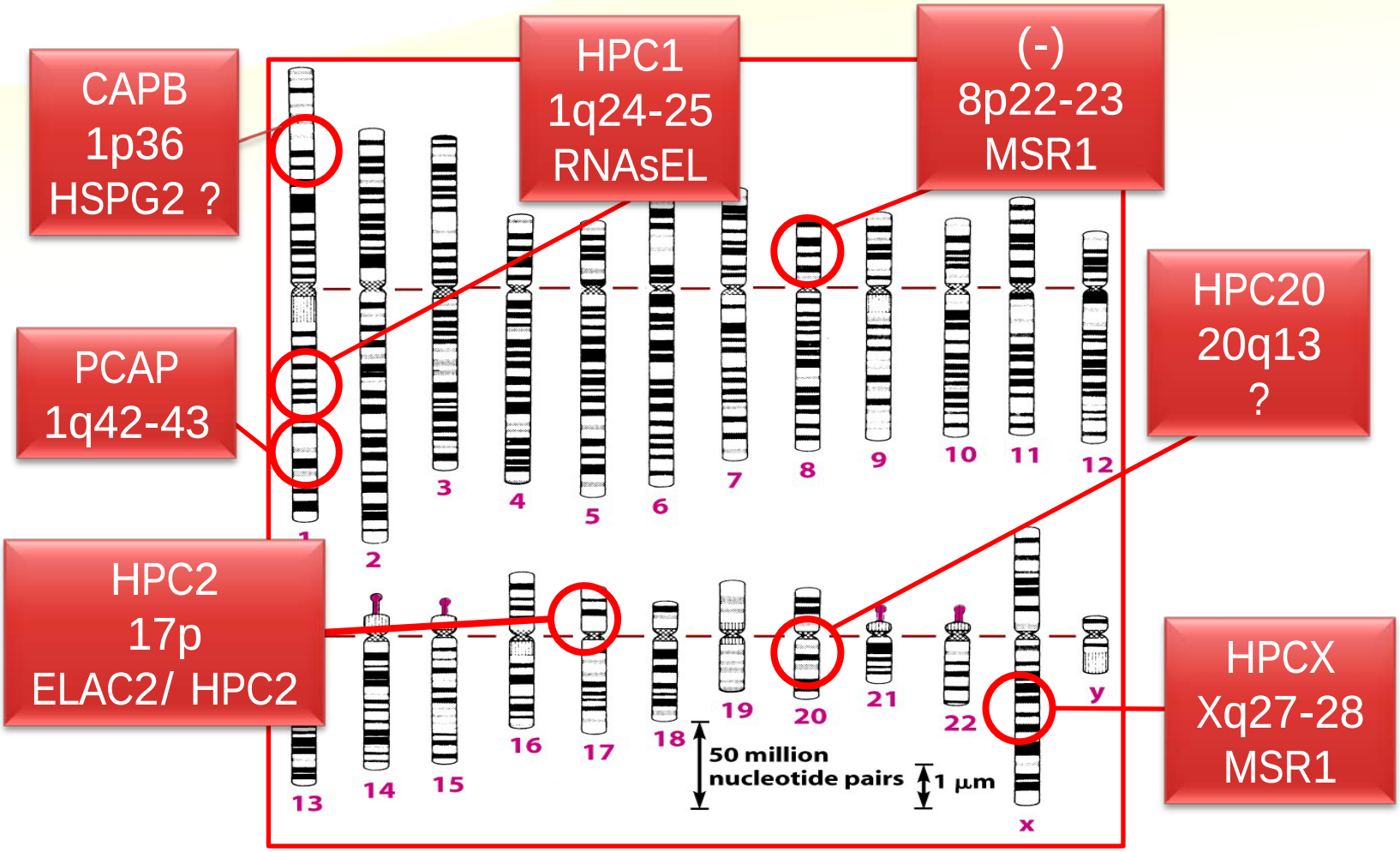
Tomonori Habuchi , M.D.
Department of Urology
Akita University Graduate Schoool of Medicine

Identification of Genetic, Epigenetic and Biological Alterations in Cancer Cells and Patients



Candidate Prostate Cancer Genes and Chromosomal Loci

- from Familial/Hereditary Prostate Cancer -



Candidate Prostate Cancer Genes *from Familial/Hereditary Prostate Cancer*

Promising, but these cases cover only 3 – 5% of all prostate cancer patients

Genome-Wide Association Study

Pateint DNA

Non-Pateint DNA

SNP Genotyping on a Whole-Genome Basis

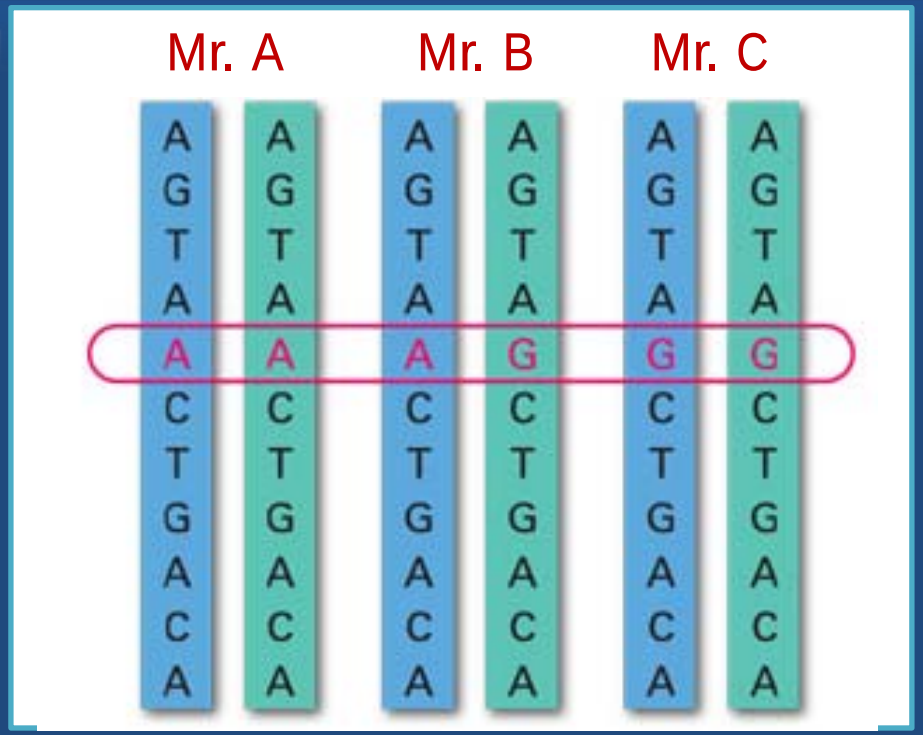
Compare SNP-Genotype and Extract Candidate Loci

Genetic Polymorphisms

DNA sequence is 99.9 % identical among individuals.
0.1 % DNA sequence variation cuases inter-individual remarkable differences.

SNP (Single Nucleotide Polymorphism)

No.= 3-10 million



Genome-Wide Association Study

Pateint DNA

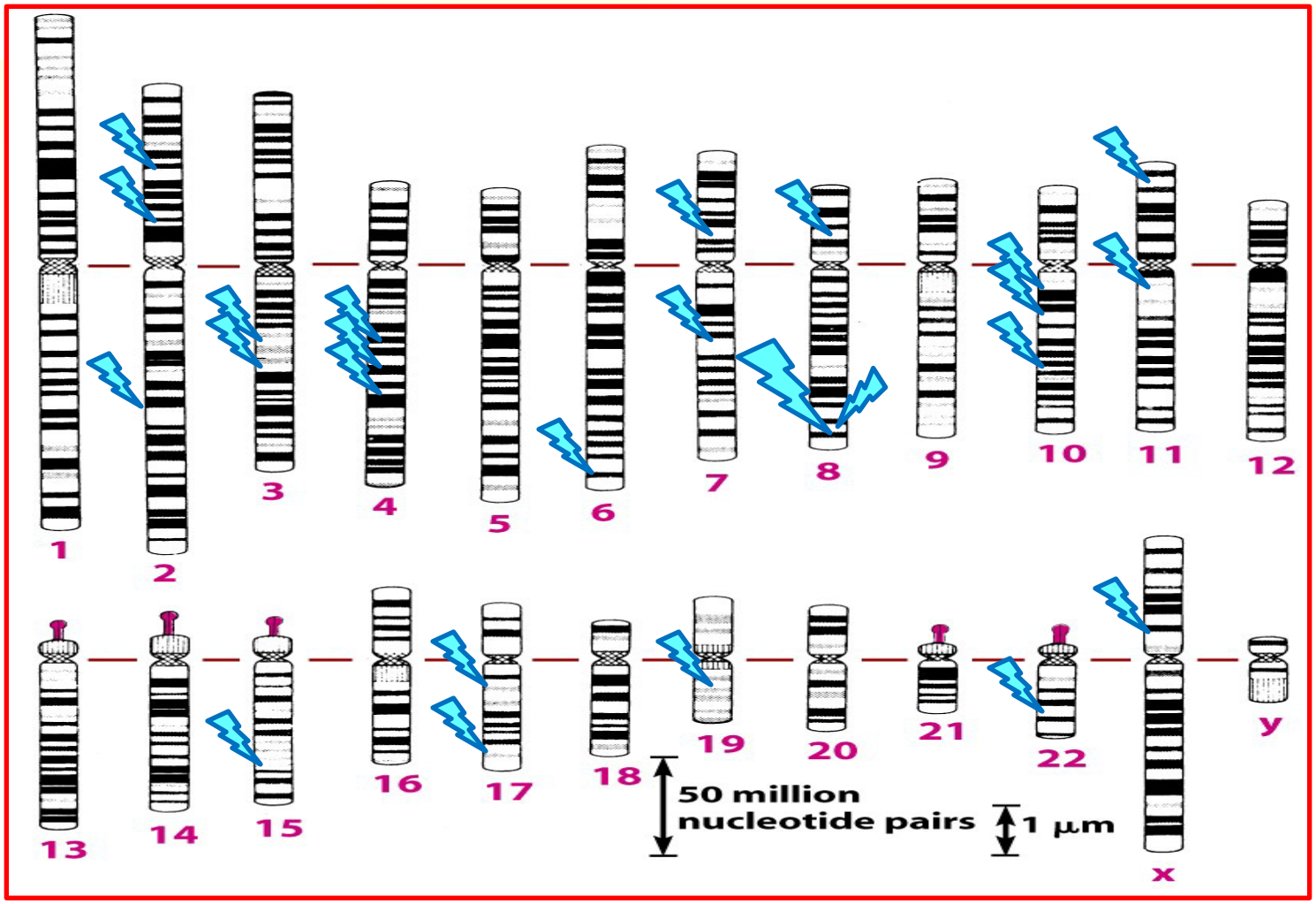
Non-Pateint DNA

SNP Genotyping on a Whole-Genome Basis

Compare SNP-Genotype and Extract Candidate Loci

Candidate Prostate Cancer Genes and Chromosomal Loci

- from Genome-Wide Association Study (GWAS) -



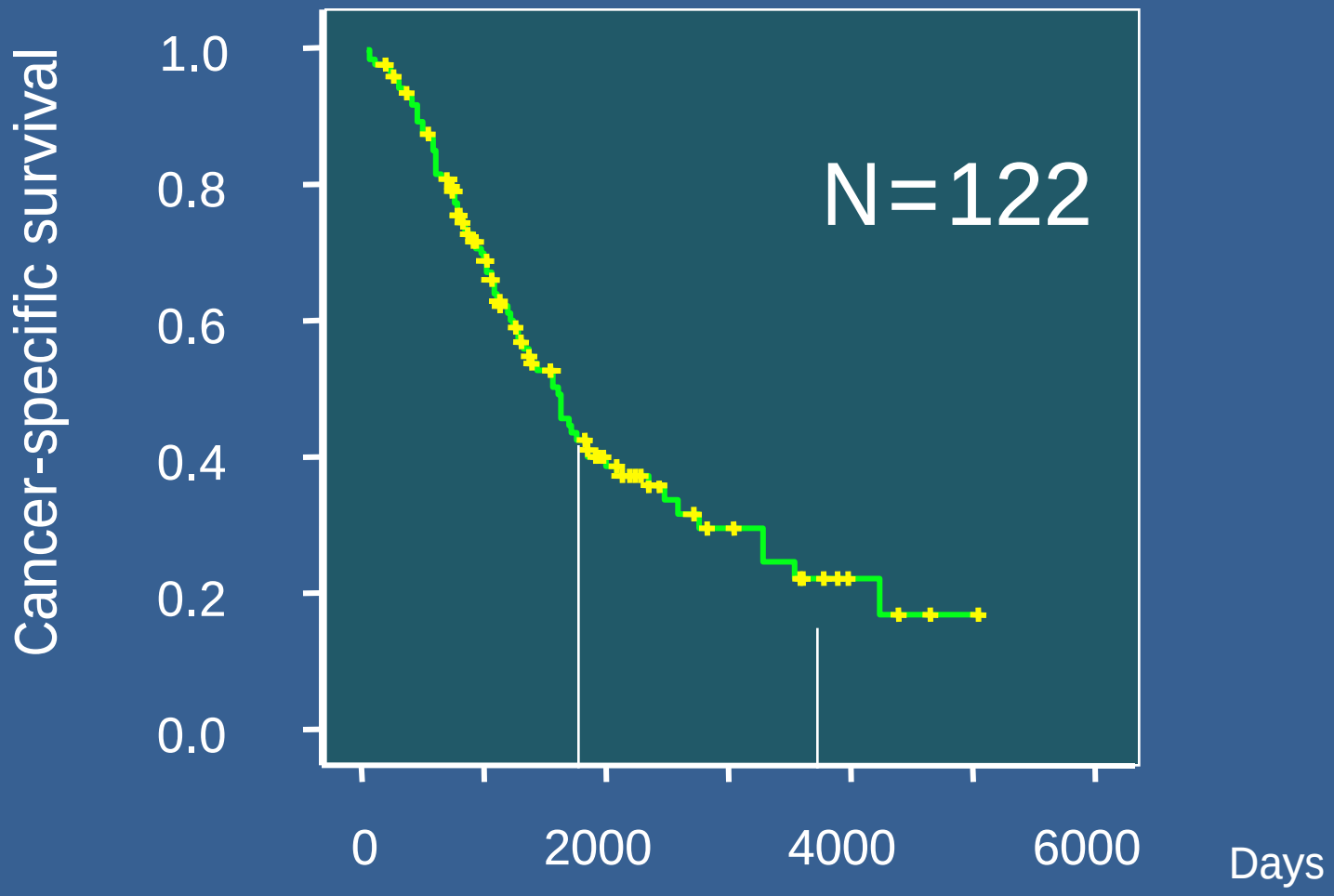
Candidate Prostate Cancer Loci *from* Genome-Wide Association Studies

Promising, but most candidate loci are associated with a higher risk below 1.3.

May be time consuming and difficult to apply the findings to the clinical settings.

DNA Polymorphism Anlysis to Identify Candidate Genes for Prostate Cancer Progression

Cancer Specific Survival in 122 Metastatic Prostate Cancer (D2) Patients



Background

- The prognosis of metastatic prostate cancer significantly differs among individuals.
- While various clinical and biochemical prognostic factors have been suggested, host genetic factors may also affect the progression and response to the treatments.
- Genetic polymorphisms may be good prognostic predictors of metastatic prostate cancer patients.

Materials and Methods

- 122 prostate cancer patients with bone metastasis at the diagnosis
  Metastasis confirmed by Bone Scan and/or CT
  Madian age=73, Median follow-up=1167 days

- 13 polymorphisms of the genes related to the steroid hormone synthesis and growth factors were genotyped.
  PCR-RFLP for SNPs, or
  GeneScan for repeat polymorphisms

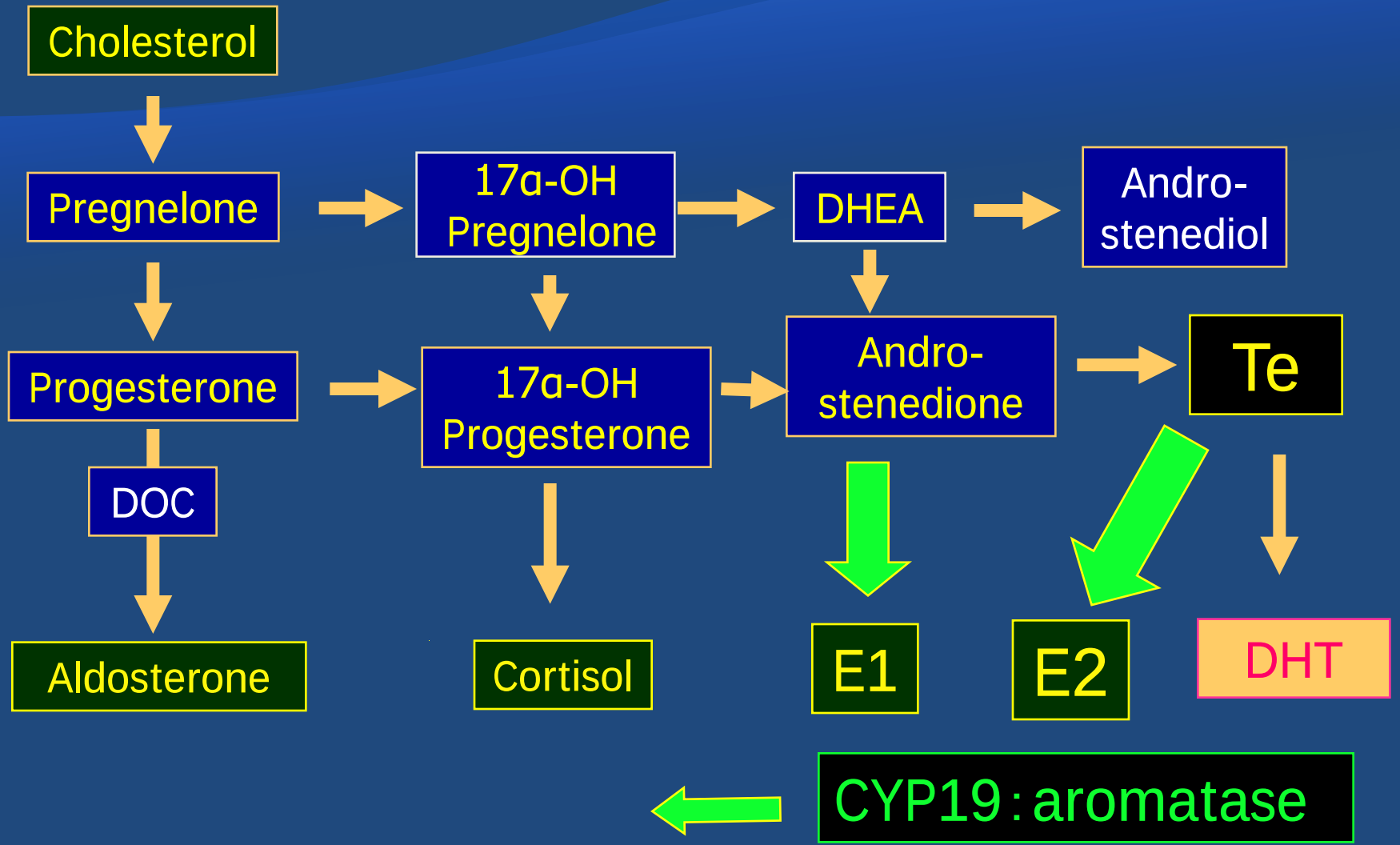
13 Polymorphisms Analyzed

Gene	Name	Type	Site	
VDR	Vitamin D receptor	SNP	*Bsm*I	3' UTR
CYP17		SNP	T-34C	promoter
SRD5A2	5-alpha reductase	SNP	V89L	exon 1
CYP11A1	Side chain cleavage	repeat	(TTTTA)n	promoter
AR	Androgen receptor	repeat	(CAG)n	exon 1
CYP19	Aromatase	repeat	(TTTA)n	intron 4
CCND1	Cyclin D1	SNP	A870G	exon 4
TGF-β1		SNP	T29C	exon 1
IGF-I	Insulin-like growth factor	repeat	(CT)n	promoter
IFGBP3	IGF binding protein	SNP	A-202C	promoter
PSA	Prostate-specific antigen	SNP	A-158G	promoter
EGF	Epidermal growth factor	SNP	G61A	exon 1
Her2/neu	ErbB-2 (HER2)	SNP	I655V	I655V

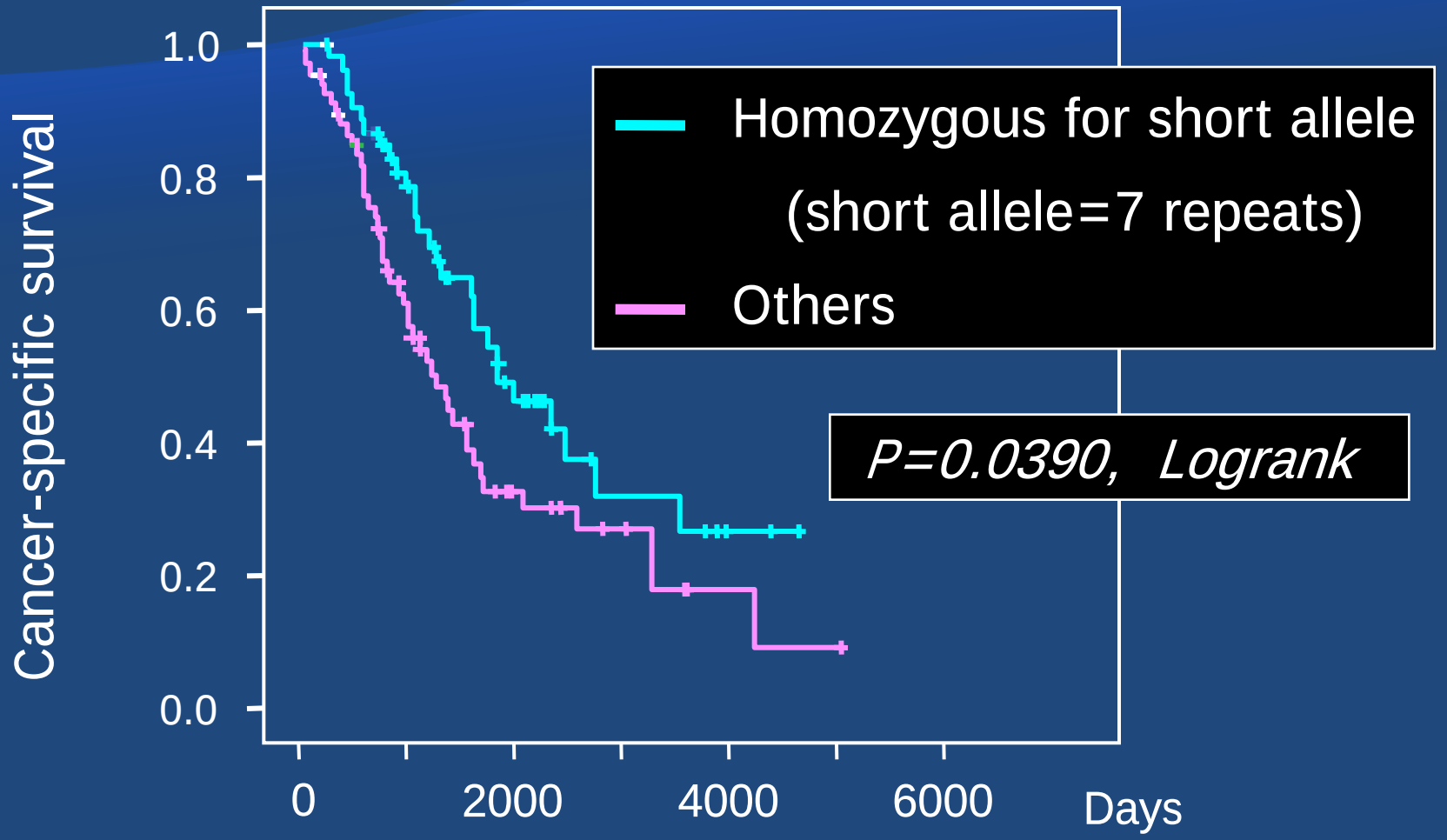
Polymorphisms with the Significant Results

Gene	Type		Site	Category
VDR	SNP	*Bsm*I	3' UTR	bb vs bB/BB
CYP17	SNP	T-34C	promoter	TT vs TC/CC
SRD5A2	SNP	V89L	exon 1	VV vs VL/LL
CYP11A1	repeat	(TTTTA)n	promoter	4 rpts vs no
AR	repeat	(CAG)n	exon 1	24 / + vs <24 rpts
TGF-β1	SNP	T29C	exon1	TT vs. TC/CC
CYP19	repeat	(TTTA)n	intron 4	7 rpts vs. others
CCND1	SNP	A870G	exon 4	AA vs AG/GG
IFGBP3	SNP	A-202C	promoter	AA vs AC/CC
IGF-I	repeat	(CA)n	promoter	=>19 rpts vs. no
PSA	SNP	A-158G	promoter	GG vs GA/AA
EGF	SNP		exon	GG vs GA/AA
Her2/neu	SNP	I655V	I655V	II vs IV/VV

Steroid Hormone Synthetic Pathway and CYP19



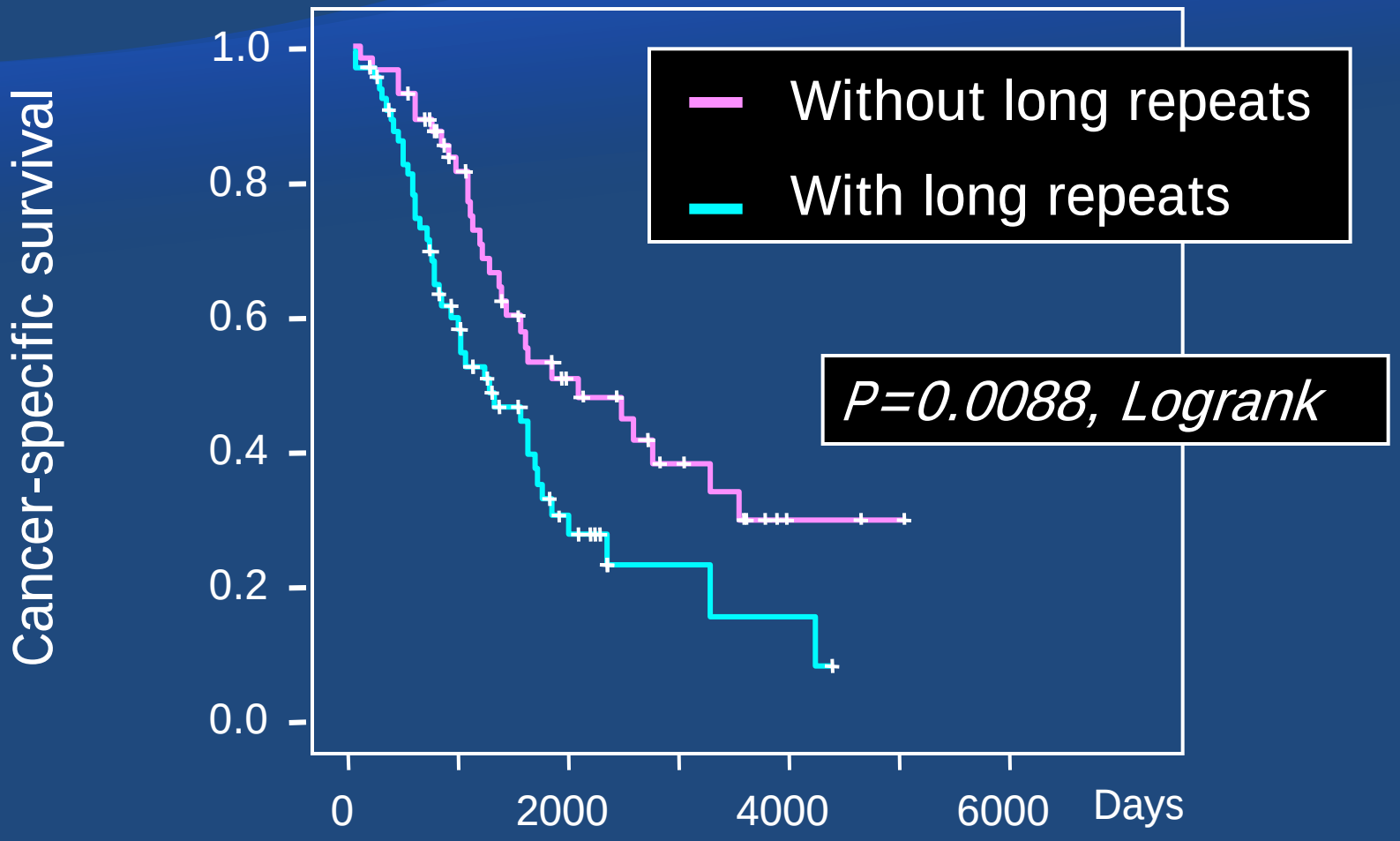
Cancer-Specific Survival and CYP19 Polymorphism



Insulin-like Growth Factor -I and Prostate Cancer

- Potent mitogen
- Anti-apoptotic and survival factor in androgen-deprived conditions
- IGFBP-2, -3, -4, and -5 block IGF-1 action
- The high serum IGF-1 level and the high IGF-1/ IGFBP-3 ratio are risk factors for prostate cancer

Cancer-Specific Survival and IGF-1 Polymorphism



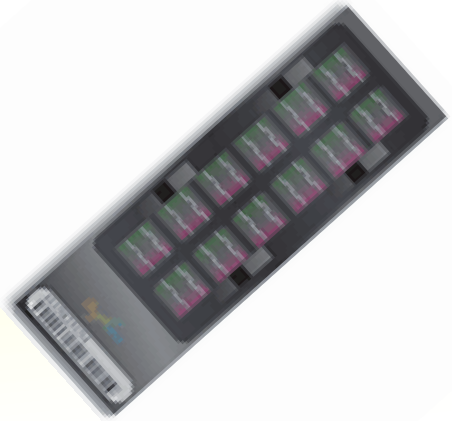
long repeats=19 repeats or more

Patients

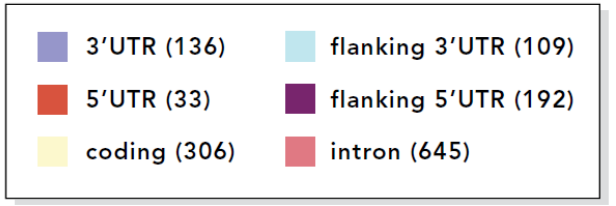
- Patients: 188 PCa patients with bone metastasis at initial diagnosis

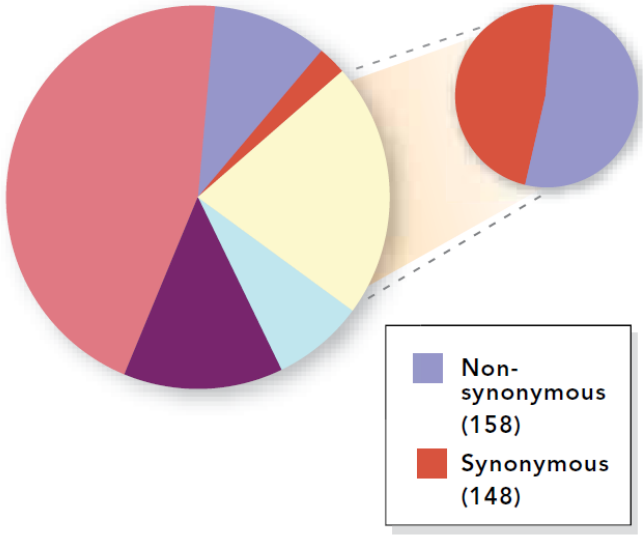
	Mean ± SD	Median
Age (yr)	69.4 ± 8.8	70 (45 - 89)
PSA (ng/ml)	1083 ± 1970	317 (0.2 - 12490)
HGB (g/dl)	13.3 ± 2.0	13.5 (6.2 – 17.4)
ALP (IU/ml)	654 ± 896	308 (7 - 5870)
LDH (IU/ml)	299 ± 183	238 (133 - 1276)
Gleason score	(%)	
7<	15 (8.0)	
7-8	72 (38.3)	
>8	188 (53.7)	

Genotyping



- Cancer SNP panel (Illumina®)
  - 408 cancer-related genes
  - 1421 SNPs selected from "NCI SNP500 Cancer Database"





Mean SNPs/gene	3.5
Median SNPs/gene	3.0
Minimum SNPs/gene	1
Maximum SNPs/gene	23
Total genes	408
Total SNPs	1421

Statistical Analyses

- SNP screening: Comparing cancer-specific survival (CSS) using dominant, recessive and additive models for each variant allele
- Validation of candidate SNPs: Developing a prognostic scoring index to classify high-risk and low-risk groups (a leave-one-out cross validation)
- Multivariate analysis: Using variables
  - Risk group, PSA, HGB, ALP, LDH, Gleason score

Results
SNP screening

- 14 SNPs in 6 genes were identified to have statistically significant association with the cancer-specific survival with cut-off level of 30% false discovery rate.

2q (PSM1, CASP8)
5q (XRCC4, IL13)
10p (GATA3)
12q (IGF1)

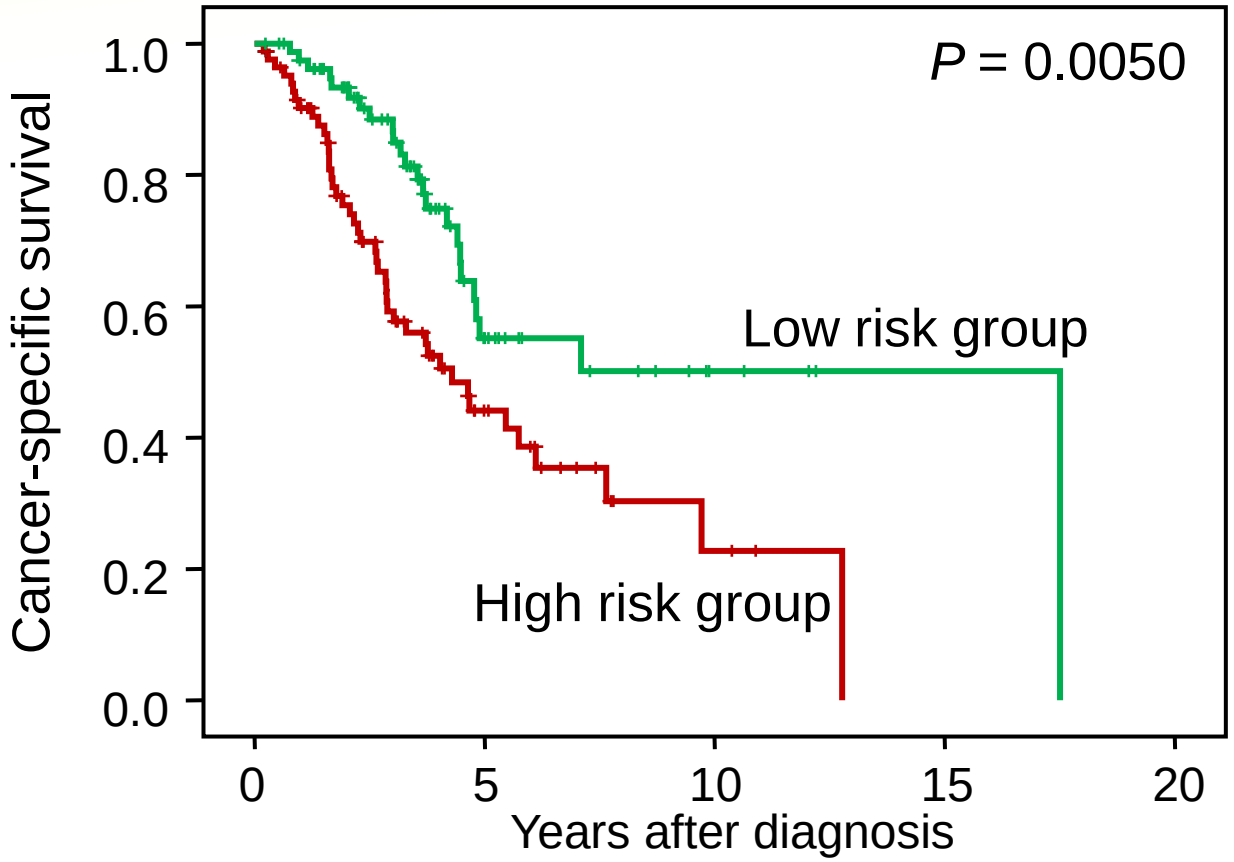
Highly Ranked SNPs

Ranking SNP	Chromosome	Gene	Function
rs2891980	5q14	XRCC4	DNA repair
rs1805377			
rs256550	2q31	PMS1	DNA mismatch repair
rs256552			
rs256564			
rs256563			
rs256567			
rs1295686	5q31	IL13	Cytokine
rs20541			
rs2162679	12q22-24	IGF1	Growth factor
rs570730	10p14	GATA3	Transcription factor
rs10752126			
rs569421			
rs2293554	2q33	CASP8	Apoptosis

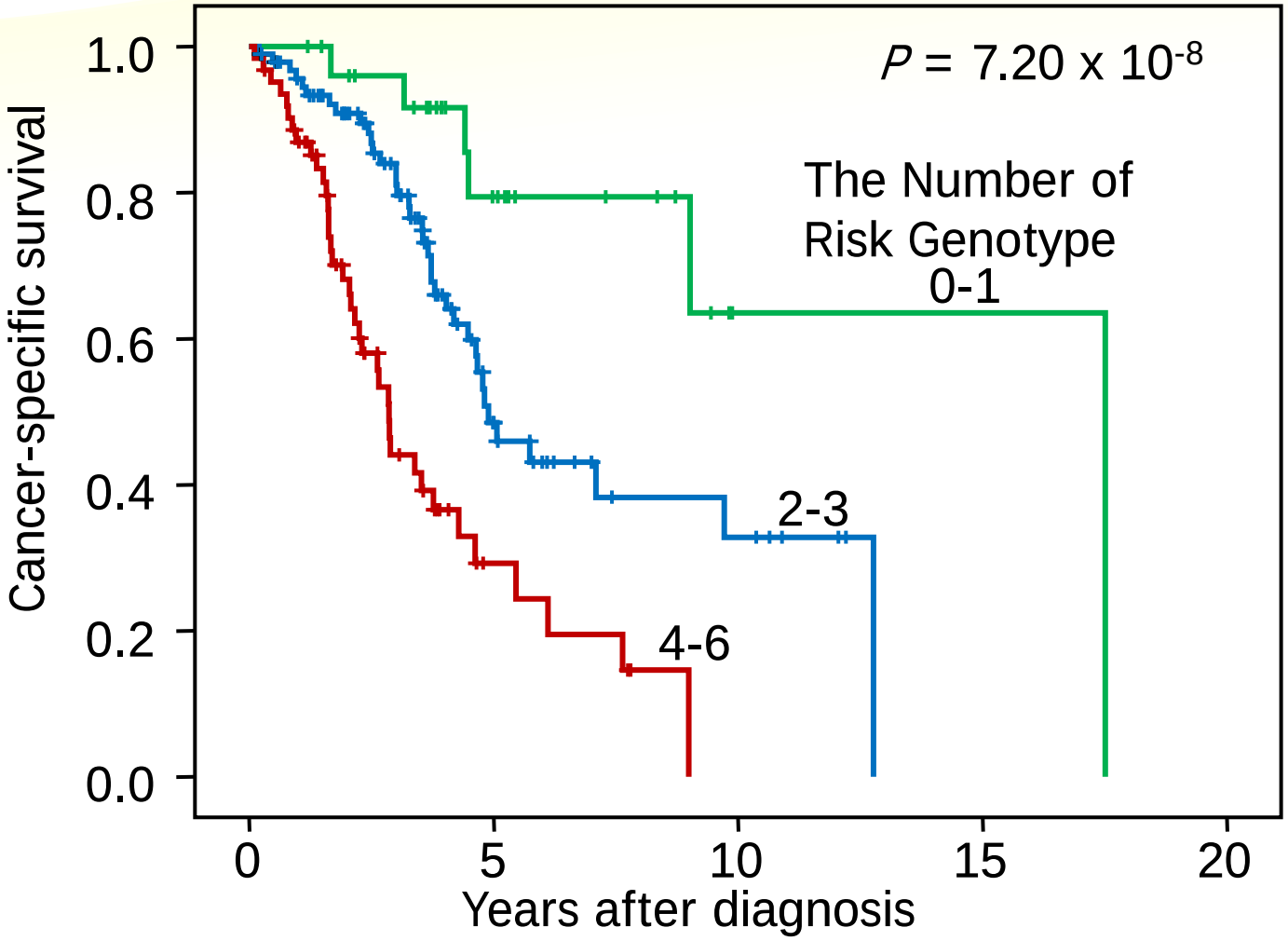
Results

Validation of candidate SNPs

- Patients were categorized into low and high risk group by a leave-one-out cross validation method for validating the SNPs



CSS of Patients Categorized According to the Number of Risk Genotype



Results

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age (≥70 vs <70)	1.32	0.224	-	-
PSA (≥315 vs <315)	1.41	0.139	-	-
HGB (≤13.5 vs <13.5)	1.13	0.617	-	-
ALP (≥350 vs <350)	2.88	2.17 E-5	2.52	5.55 E-4
LDH (≥500 vs <500)	2.69	1.39 E-3	1.74	0.0897
GS (≥9 vs <9)	2.38	2.47 E-4	1.23	5.78 E-3
# of Risk Genotype (4-6 vs 0-3)	**3.22**	**4.67 E-5**	**3.23**	**1.63 E-4**

Summary

- 14 SNPs in 6 genes were identified to have statistically significant association with the CSS.
- The predicting model using the SNPs showed a statistically significant cross-validated accuracy in predicting high- and low-risk groups on the CSS.
- The model may be promising for accurately predicting the outcome and optimizing the individualized treatment in metastatic PCa patients.

Toward the Identification of New Candidate Molecular Targets for Prostate Cancer Progresssion

High-fat Diet Associated Prostate Cancer Progression and Candidate Genes

The association between obesity and prostate cancer is complex and uncertain…….

Freedland SJ and Platz EA: Epidemiologic Reviews 2007

Obesity and Prostate Cancer: Making Sense out of Apparently Conflicting Data

By reviewing 22 prospective studies and 3 recent large studies, it is suggested that

"Obesity may reduce the risk of nonaggressive disease while it may promote aggressive disease."

Obesity and Prostate Cancer Progression

Impact of Obesity on Biochemical Control After Radical Prostatectomy for Clinically Localized Prostate Cancer: A Report by the Shared Equal Access Regional Cancer Hospital Database Study Group

Stephen J. Freedland, William J. Aronson, Christopher J. Kane, Joseph C. Presti Jr, Christopher L. Amling, David Elashoff, and Martha K. Terris

JCO 2004

BMI=35 or more : An independent risk factor for PSA recurrence

170cm, 101Kg

Pathologic Variables and Recurrence Rates As Related to Obesity and Race in Men With Prostate Cancer Undergoing Radical Prostatectomy

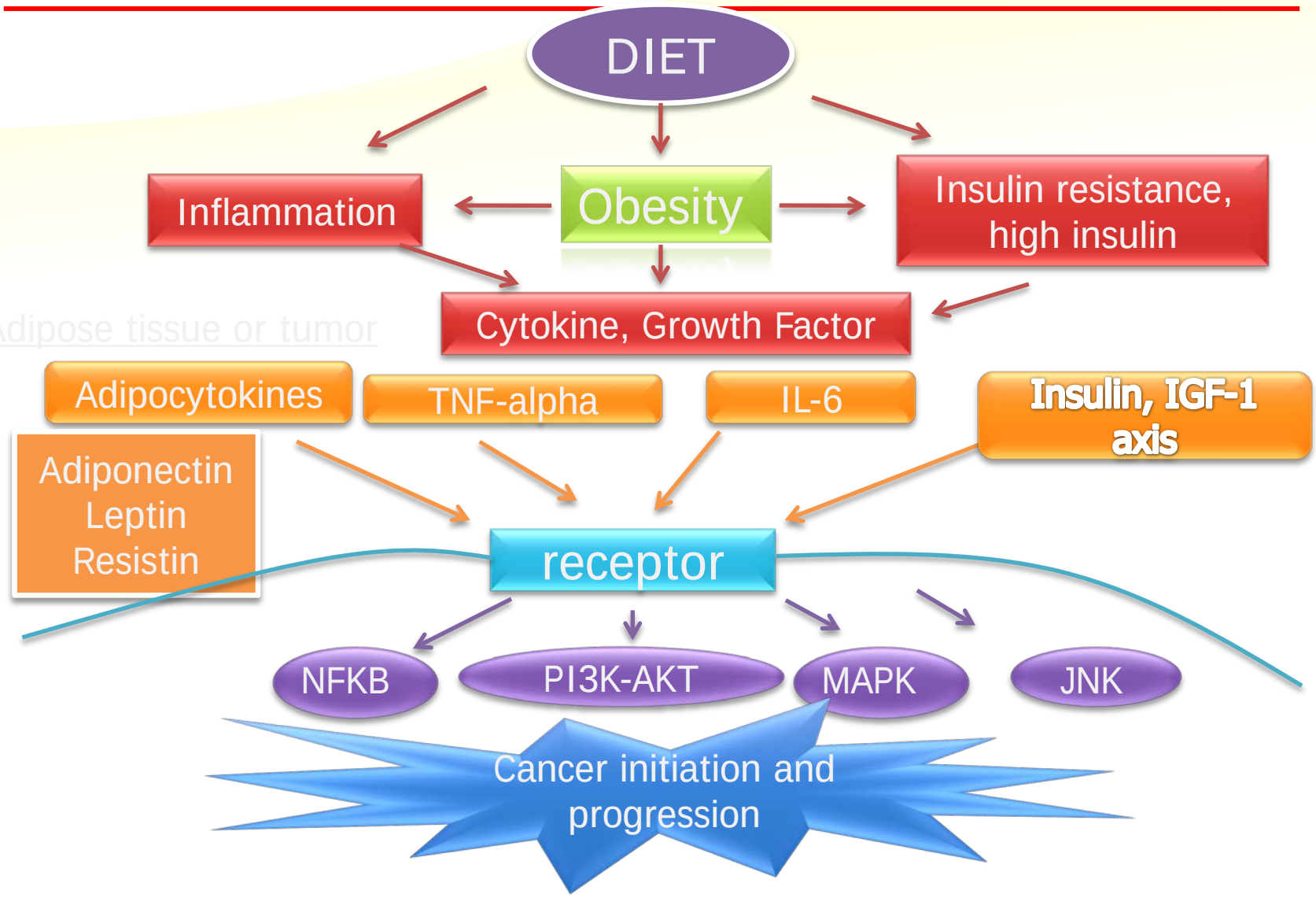
Christopher L. Amling, Robert H. Riffenburgh, Leon Sun, Judd W. Moul, Raymond S. Lance, Leo Kusuda, Wade J. Sexton, Douglas W. Soderdahl, Timothy F. Donahue, John P. Foley, Andrew K. Chung, and David G. McLeod

JCO 2004

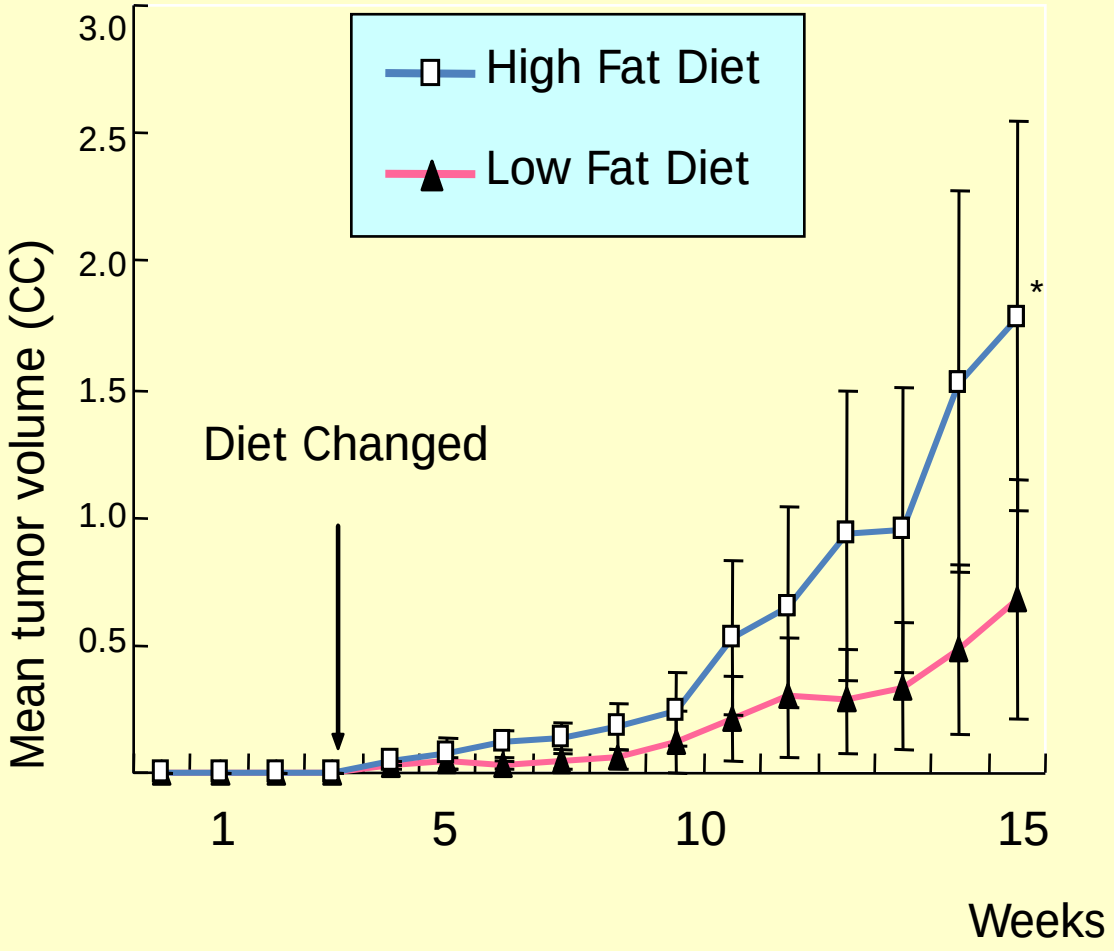
BMI=30 or more : An independent risk factor for PSA recurrence

170cm, 87Kg

Cytokines and Growth Factors Affecting Diet Induced Carcinogenesis



LNCaP Xenograft Growth under High Fat and Low Fat Diet



High Fat Diet

Low Fat Diet

Narita, Prostate 2008

Representative 9 Genes with More than 2 fold Increase in mRNA Levels by High Fat Diet

Symbol	Name	Fold Increase
MAT1A	Methionine Adenosyltransferase I, alpha	7.36
SLC5A6	Sodium-dependent Vitamin Transporter	4.67
HYOU	Hypoxia Up-regulated 1	4.63
MMP16	Matrix Metalloproteinase 16	4.46
Fn14	**TNF Receptor Superfamily, member 12A**	**3.98**
WISP1	WNT1 inducible Signaling Pathway Protein 1	3.80
JTB	Jumping Translocation Breakpoint	3.66
IGF-IR	Insulin-like Growth Factor 1 Receptor	3.43
PMP22	Peripheral Myelin Protein 22	2.52

64 genes were identified by two independent microarray experiments

Narita, Prostate 2008

The TWEAK-Fn14 signaling pathway

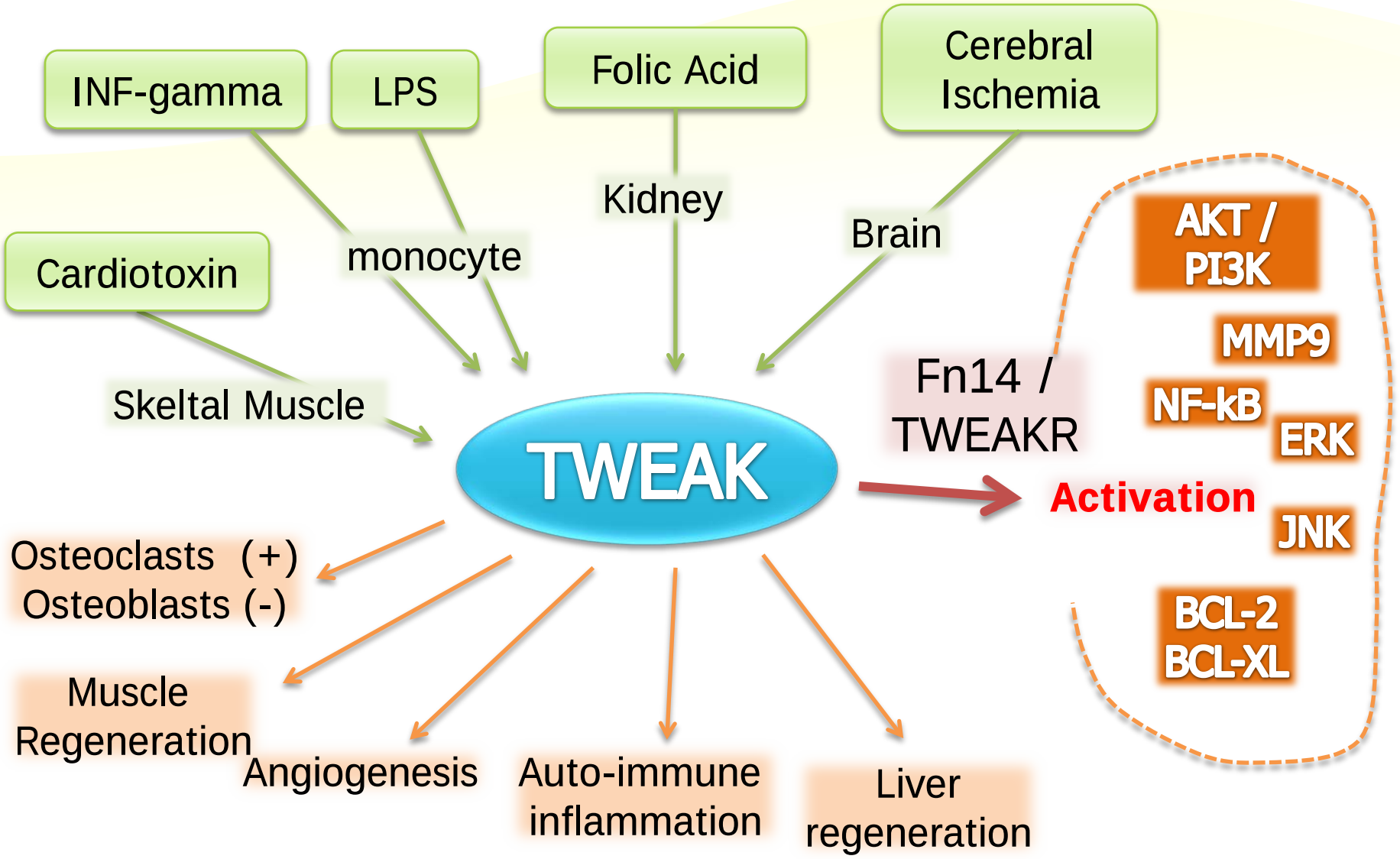
Fn14:
Fibroblast Growth Factor Inducible-14

TWEAK receptor (TWEAKR)/**Fn14**, is a TNF receptor superfamily member

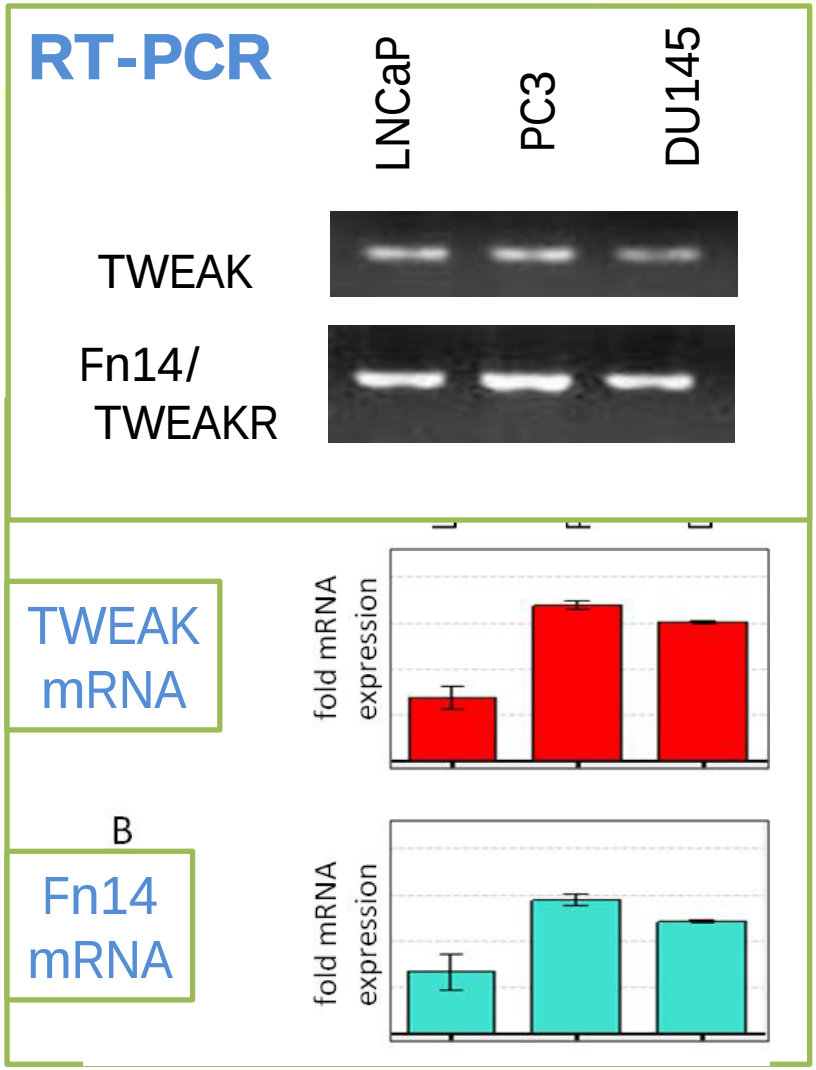
TWEAK :
*T*umor necrosis factor-like *weak* inducer of apoptosis

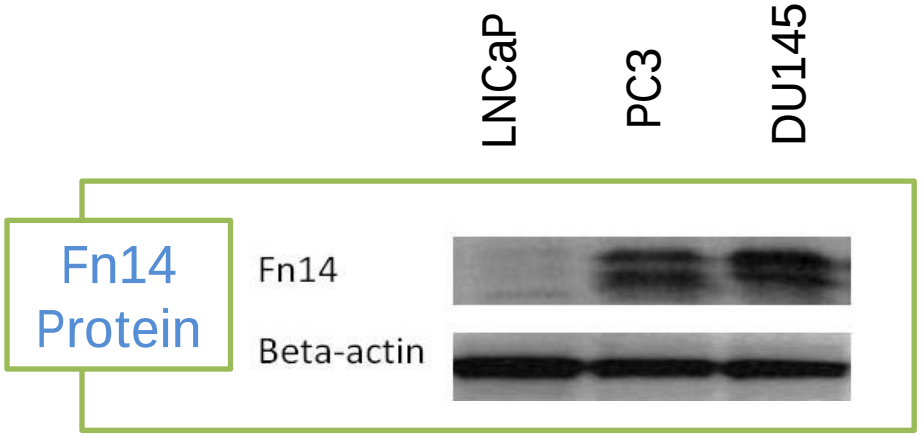
A member of the TNF ligand superfamily

TWEAK: not just a weak inducer of apoptosis



Expression of TWEAK and Fn14/TWEAKR in Prostate Cancer Cell Lines

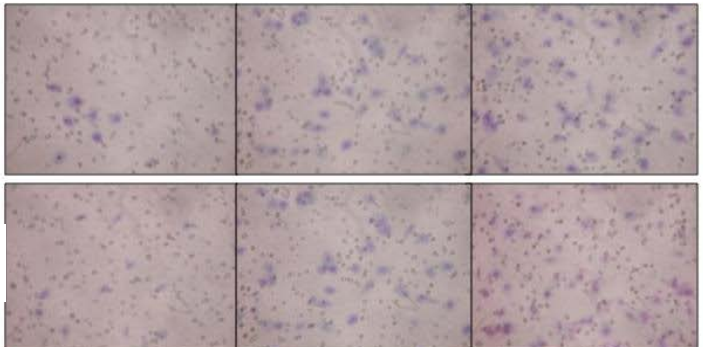




The TWEAK-Fn14 system regulates invasive capacity in PC-3 and DU145 cells

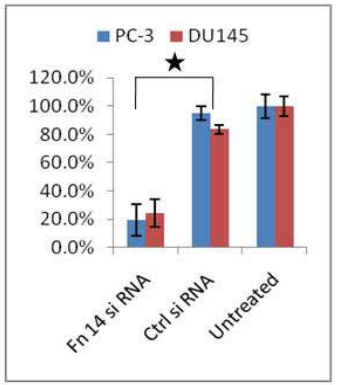
Matrigel invasion assay

A



Fn14 siRNA Ctrl siRNA Untreated

B



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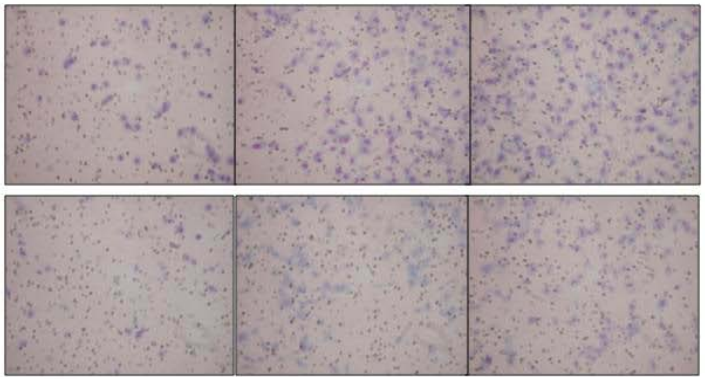
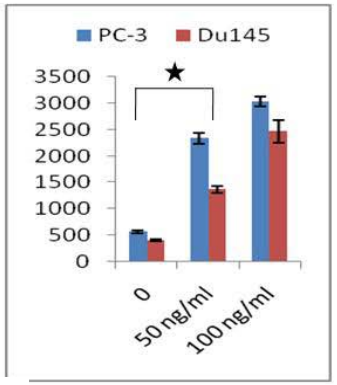
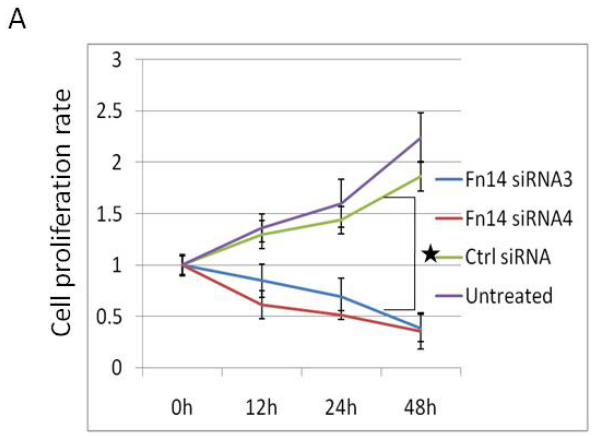


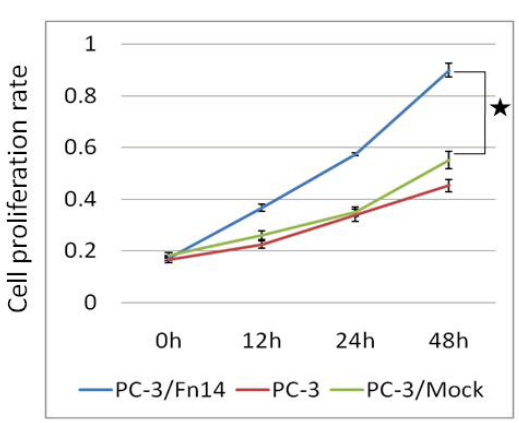
rTWEAK 0 50 100 ng/ml

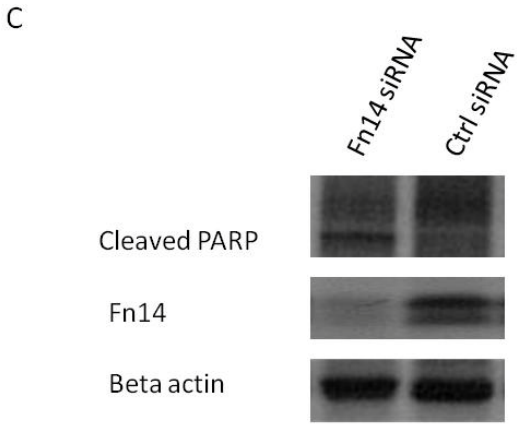
D

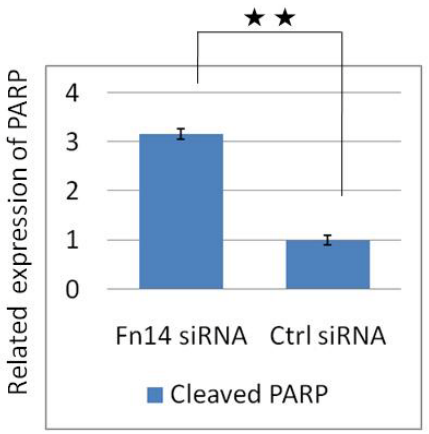


The Effect of Fn14 on Proliferation and Apoptosis in PC-3 cells



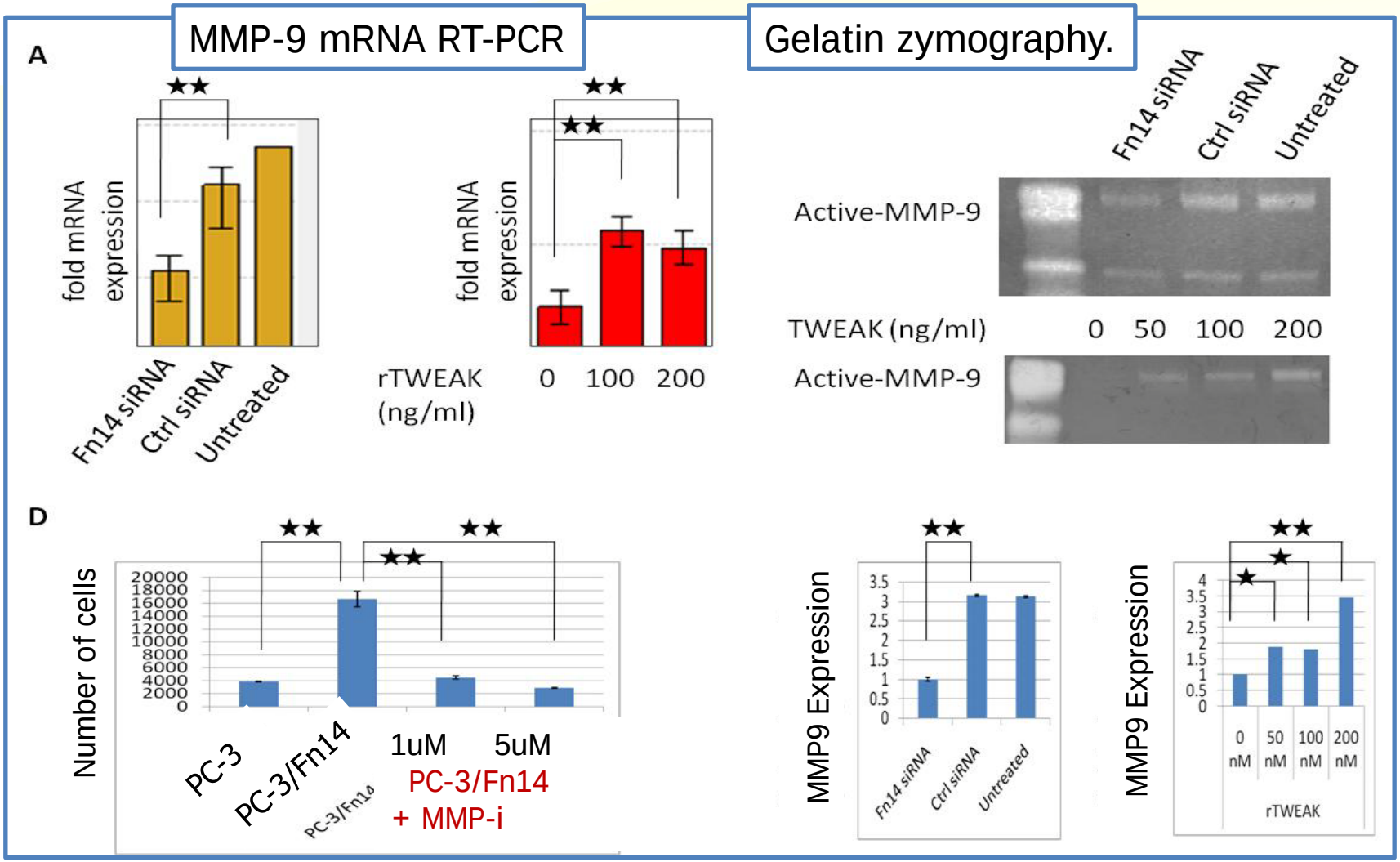




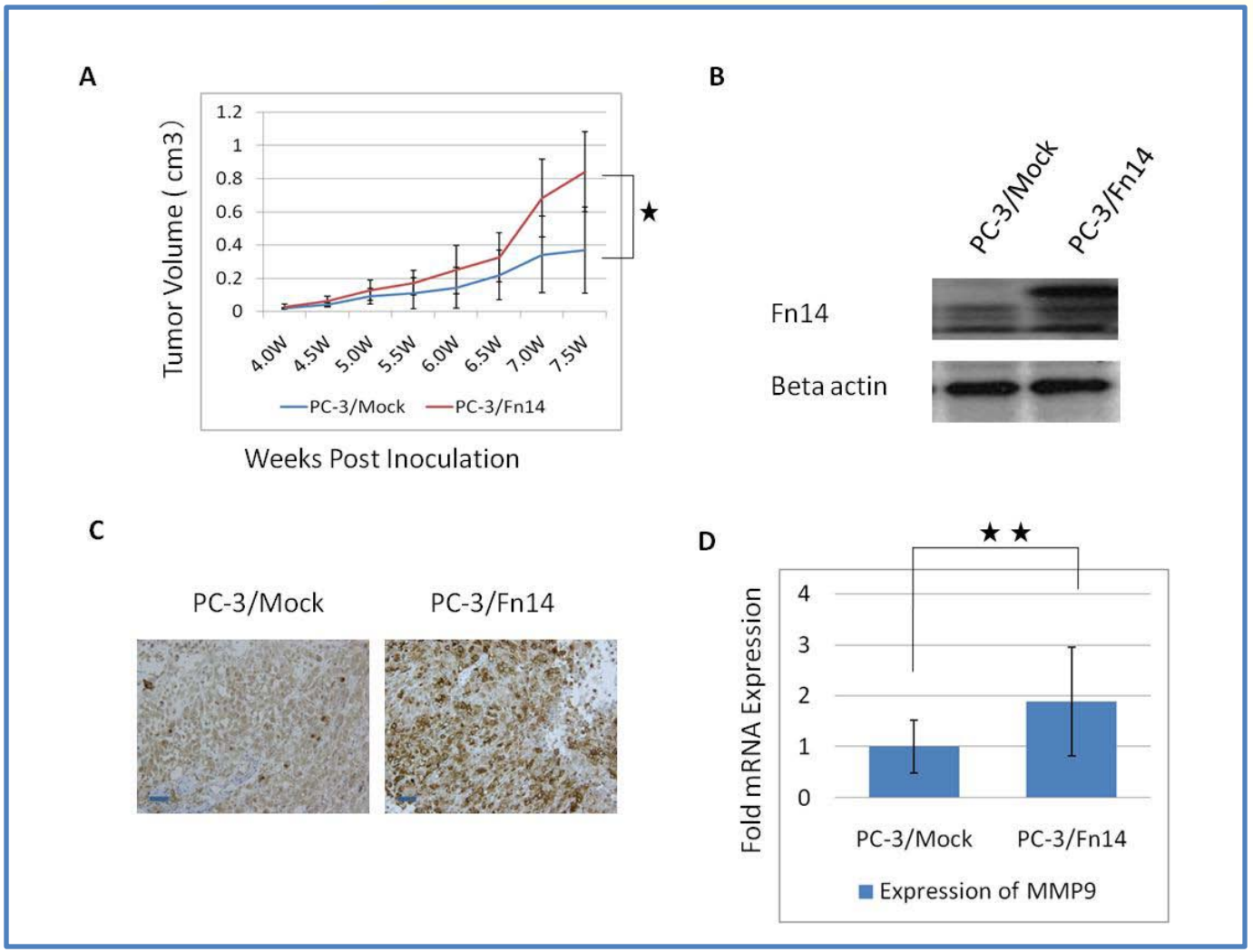


***PC-3/Fn-14 : PC-3 cells stably transfected with Fn14 cDNA*

Modulation of Invasive Capacity by the TWEAK-Fn14 system through MMP-9 Activation

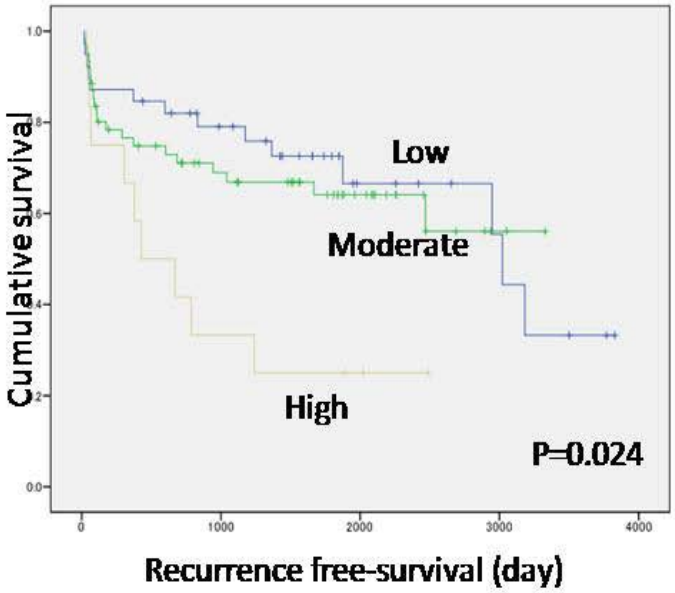


PC-3 and PC-3/Fn14 Xenograft Tumor Progression and MMP9 Expression *in vivo* Model



High Expression of Fn14/TWEAKR and Poor Outcome after Radical Prostatectomy

A



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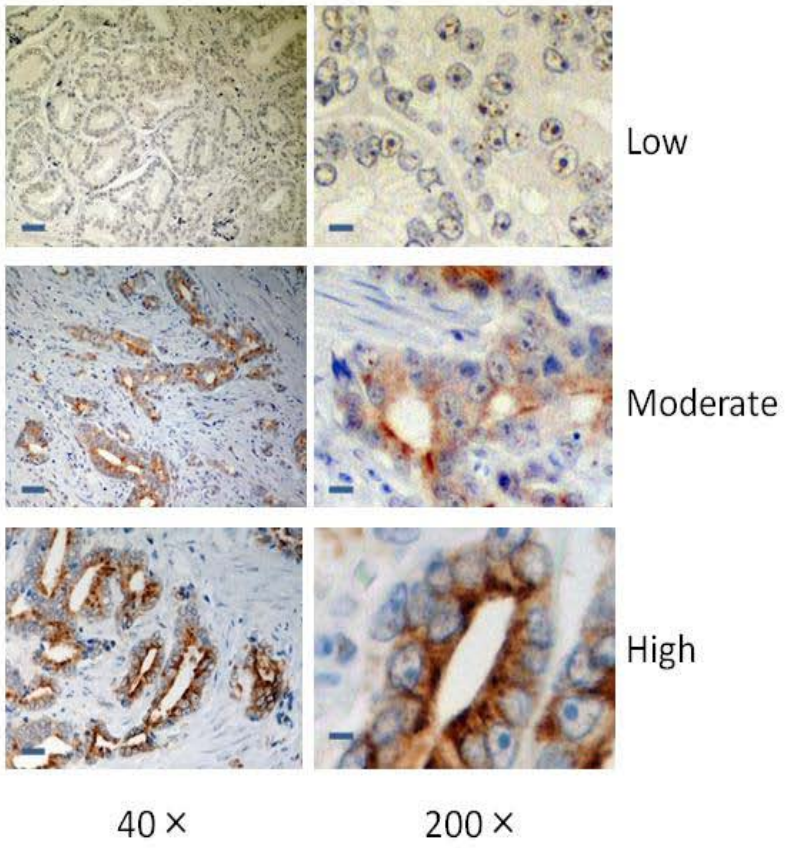
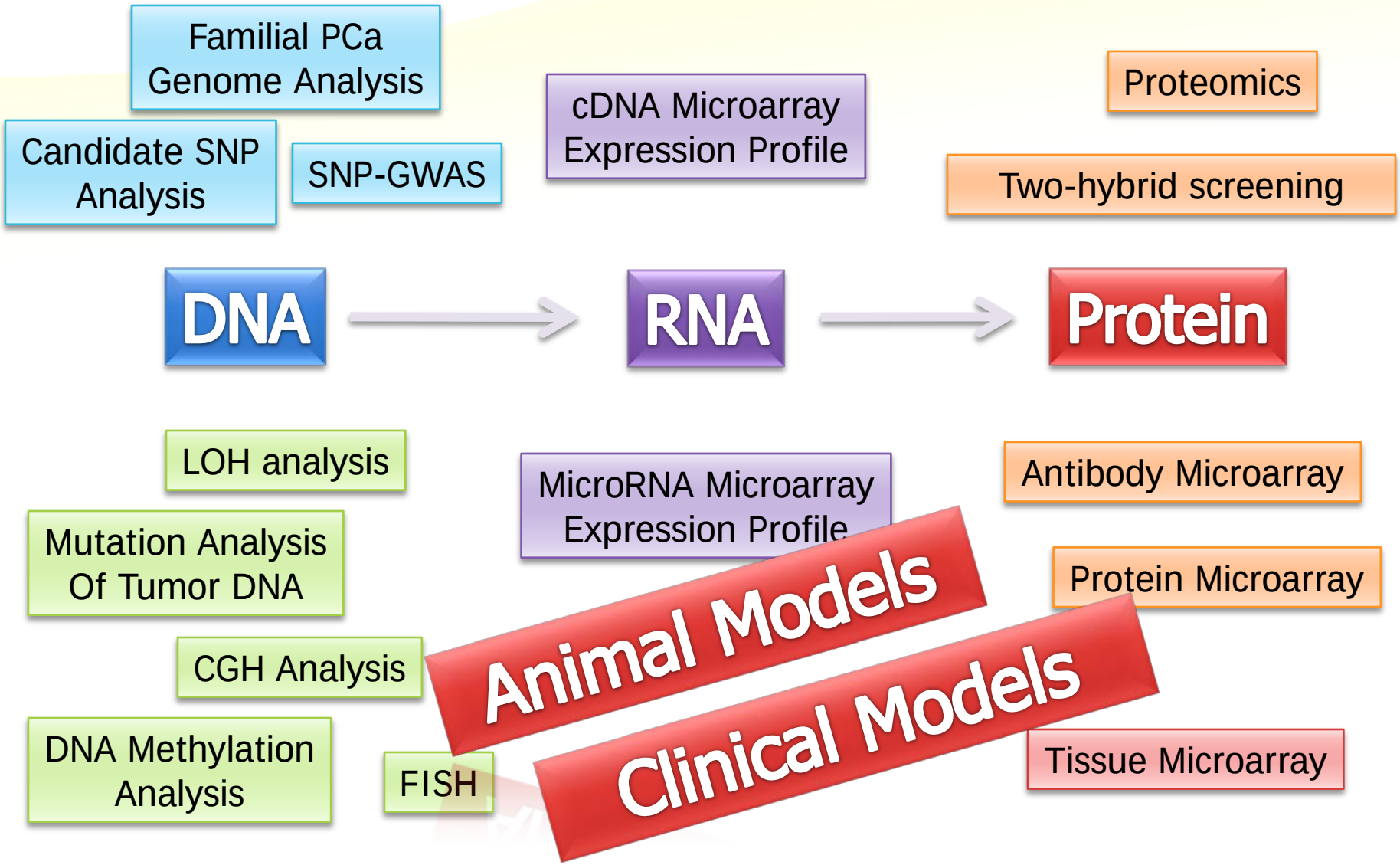


Conclusion

1) Both Fn14 and TWEAK were expressed in prostate cancer cells.
2) Fn14 expression was enhanced by TWEAK.
3) The TWAEK-Fn14 system enhanced the proliferation and tumor invasiveness of PCa cells
4) The TWAEK-Fn14 system enhanced invasiveness partly through MMP9 activation.
5) The high expression of Fn14 correlated with a poor patient outcome after radical prostatectomy.
6) The TWEAK-Fn14 system may be a potential target of prostate cancer therapy.
7) The relation between high fat diet and Fn14 activation in the progression of PCa remains to be elucidated.

Identification of New Candidate Molecular Targets



Contributers

Akita University
Norihiko Tsuchiya
Yohei Horikawa
Shintaro Narita
Takamitsu Inoue

Hirosaki University
Chikara Ohyama
Shingo Hatakeyama

Tohoku University
Youichi Arai
Seiichi Saito

Kyoto University
Osamu Ogawa
Toshiyuki Kamoto
Hiroyuki Nishiyama

Chiba University
Tomohiko Ichikawa
Hiroyoshi Suzuki

Fn14 /TWEAKR and the TWEAK-Fn14 System