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Disruption of Fibroblast Growth Factor Receptor (FGFR) Signaling as an Approach to Prostate Cancer Gene Therapy

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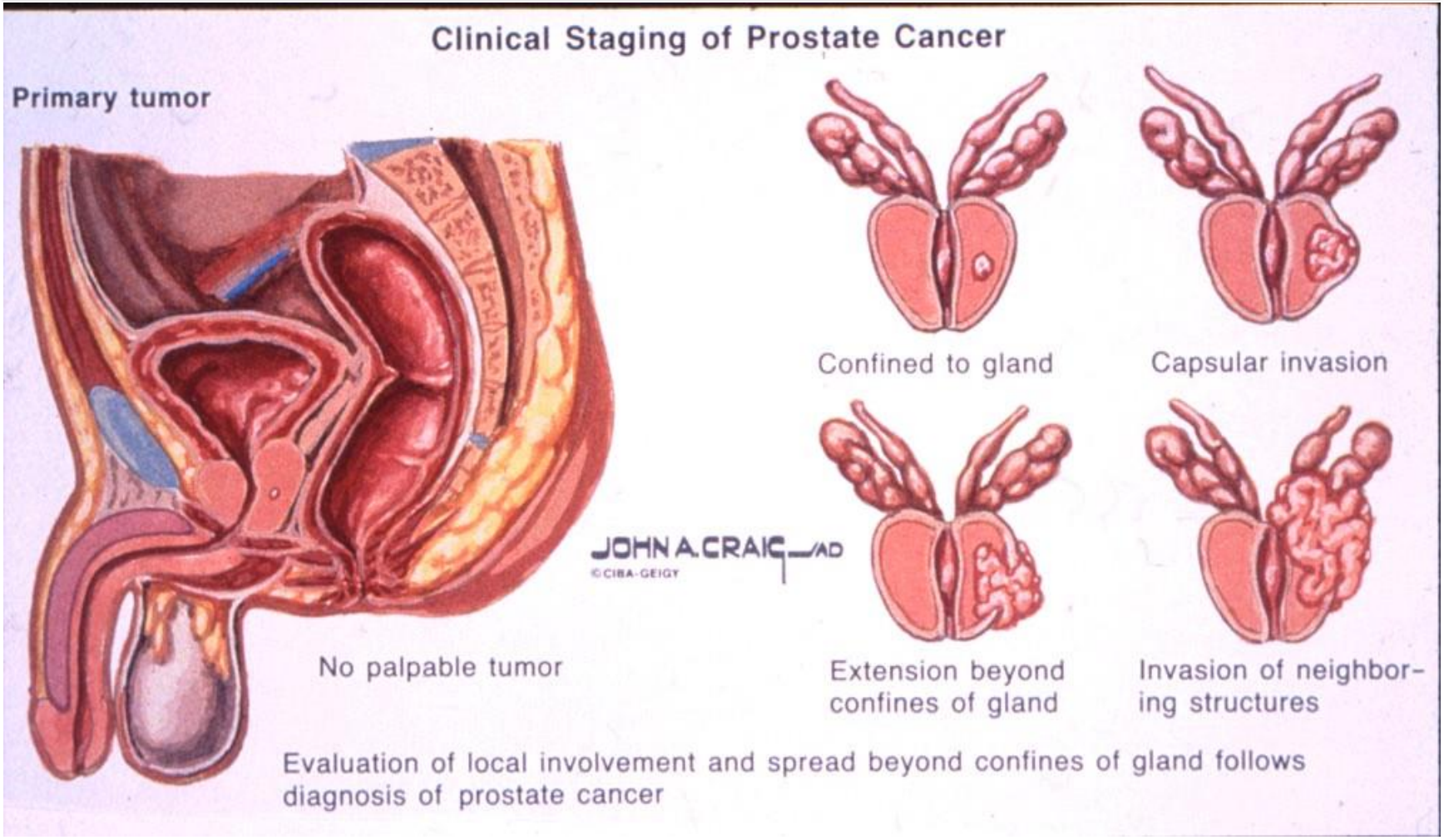
Clinical Staging of Prostate Cancer
Primary tumor
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No palpable tumor
Confined to gland
Capsular invasion
Extension beyond confines of gland
Invasion of neighboring structures
Evaluation of local involvement and spread beyond confines of gland follows diagnosis of prostate cancer

- **Fibroblast growth factor (FGF) family:**
  - **includes at least 22 different genes (FGF1-22) encoding related polypeptide mitogens.**
  - **can stimulate the proliferation of a wide variety of cells.**
  - **are expressed abundantly in prostate tissue.**
  - **are required for prostate cells to grow *in vitro*.**

- **FGF Receptors:**

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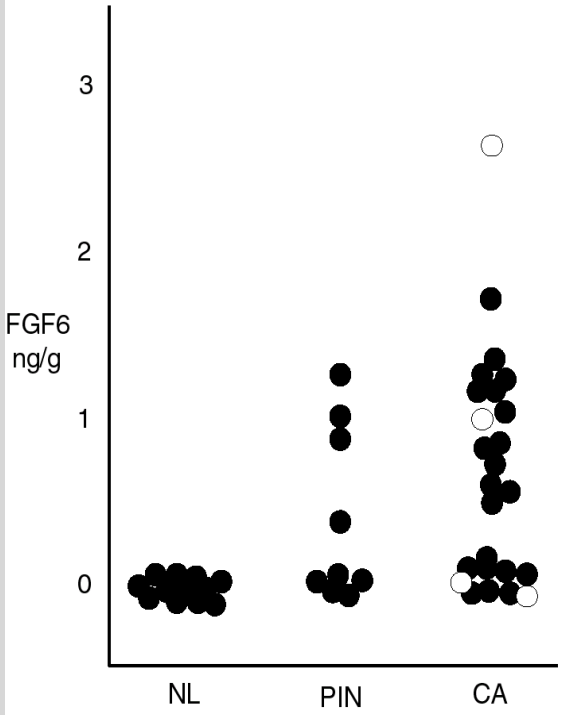
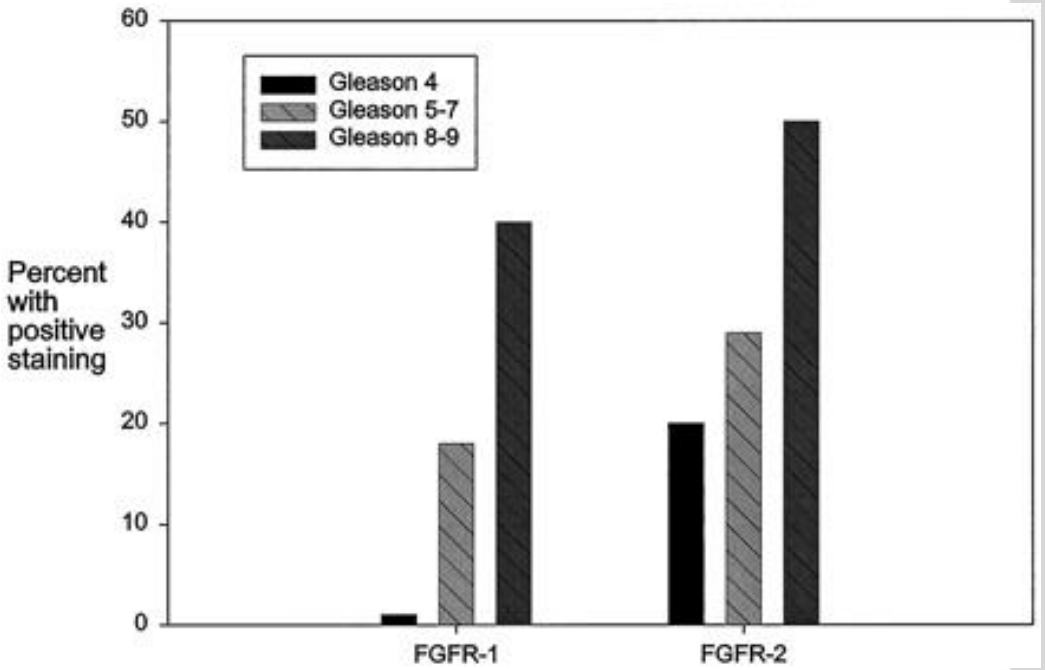
  - **located in cell membrane**
  - **bind FGFs and mediate the action of FGFs.**
  - **include four high affinity tyrosine kinase receptors (FGFR1-4).**
  - **involved in the regulation of cell growth, development, and differentiation in a variety of tissues.**
  - **have increased tyrosine kinase activity in response to binding FGF**
  - **work in pairs at cell surface**

HYPOTHESIS

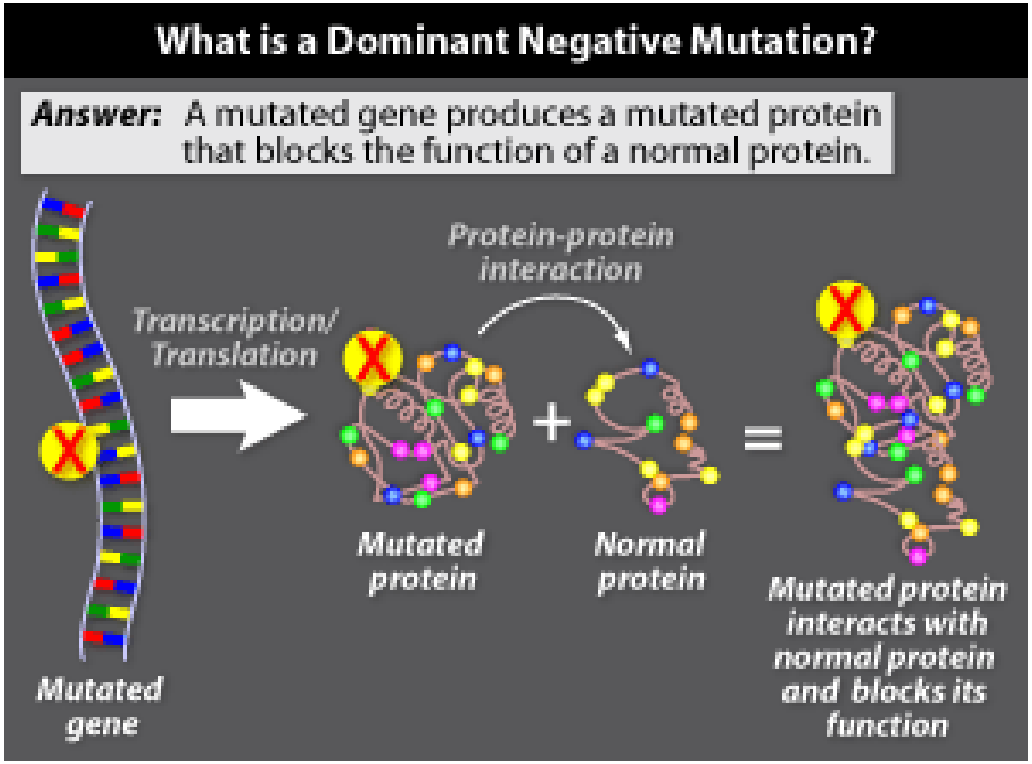
- **Prostate cancer cells are dependent upon FGFR signaling for survival and proliferation.**
- **Disruption of this signaling pathway by expression of a DN FGFR protein might contribute to death of cancer cells and can be used as gene therapy adjuvant to current treatment options.**
- **Analysis of gene expression profile in DN FGFR transfected cells might help our understanding of how DN FGFR works and the differentially expressed genes determined by microarray analysis can be used as targets for prostate cancer therapy.**

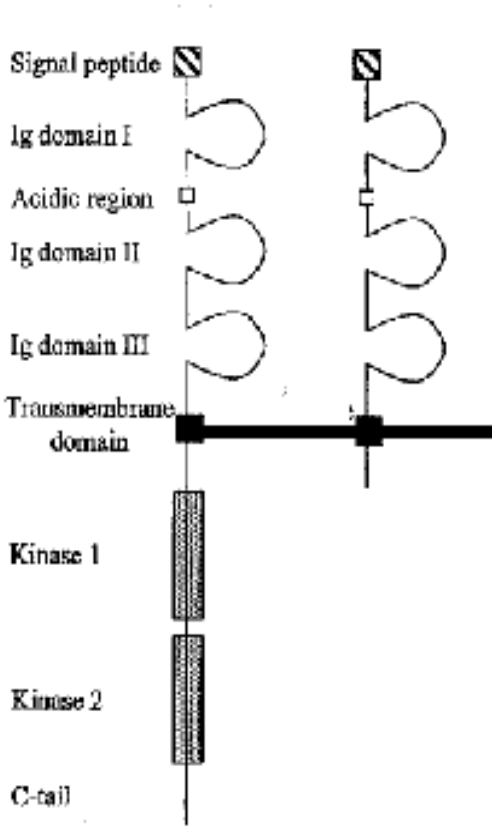
FGFs and their receptors are increased in prostate cancer





Dominant negative FGF receptor





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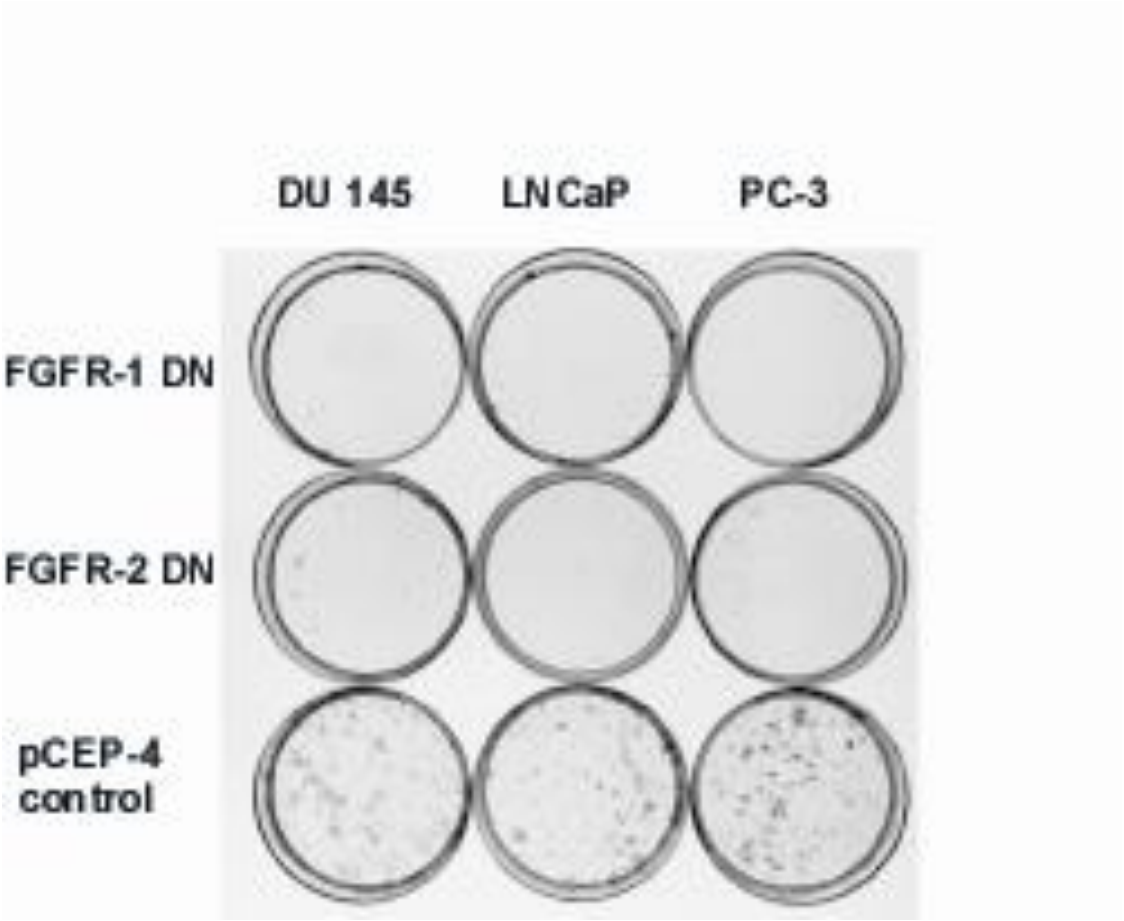
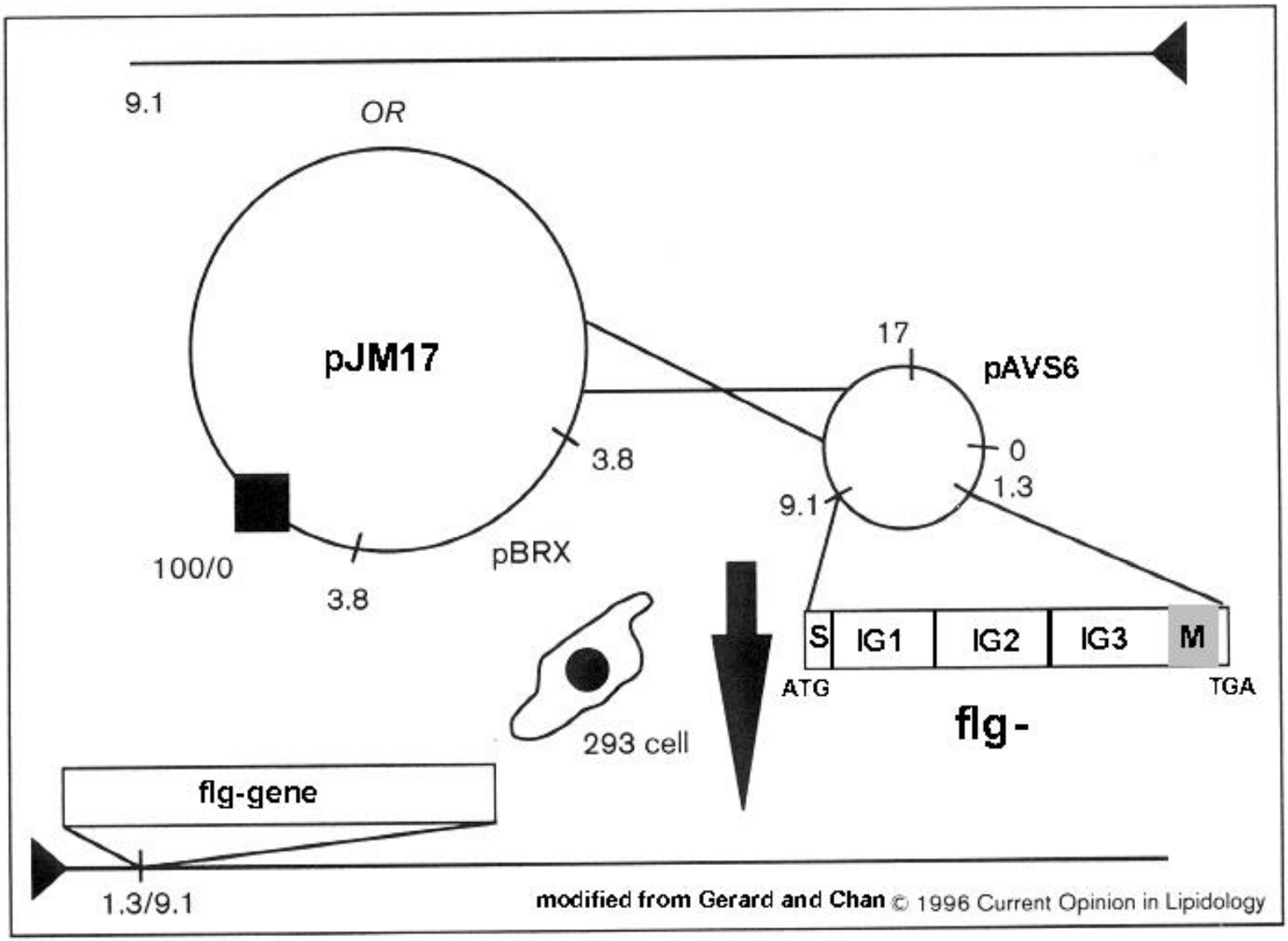
COLONY FORMATION




9.1
OR
pJM17
3.8
100/0
3.8
pBRX
17
pAVS6
0
1.3
9.1
S
IG1
IG2
IG3
M
ATG
TGA
flg -
293 cell
flg-gene
1.3/9.1
modified from Gerard and Chan © 1996 Current Opinion in Lipidology

FGFR DN markedly decreases phosphorylated FGFR-1

IP with α-PT, probed with α-FGFR1

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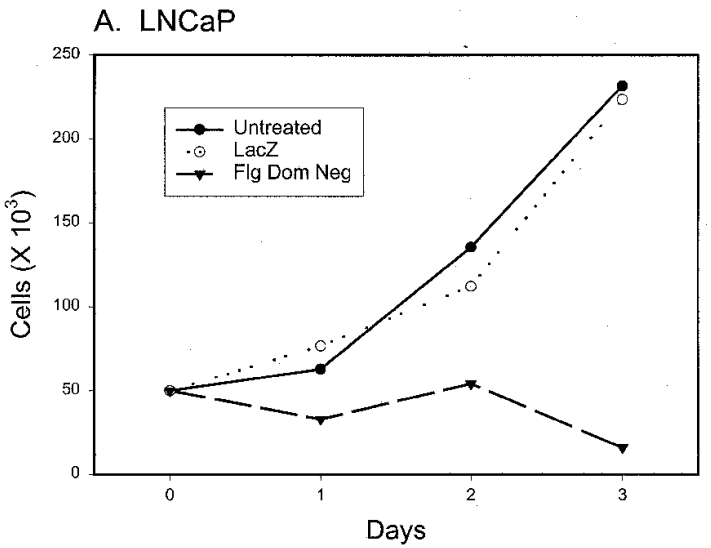
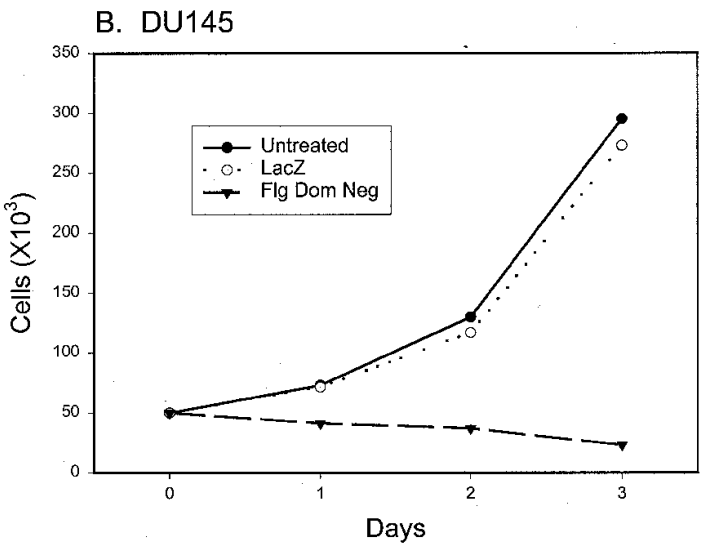
FGFR DN inhibits proliferation of prostate cancer cells





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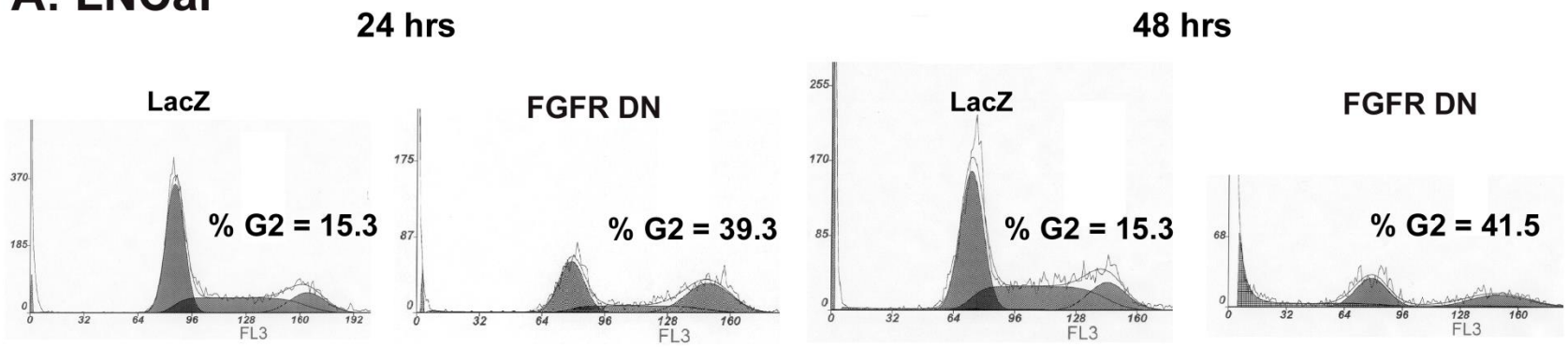
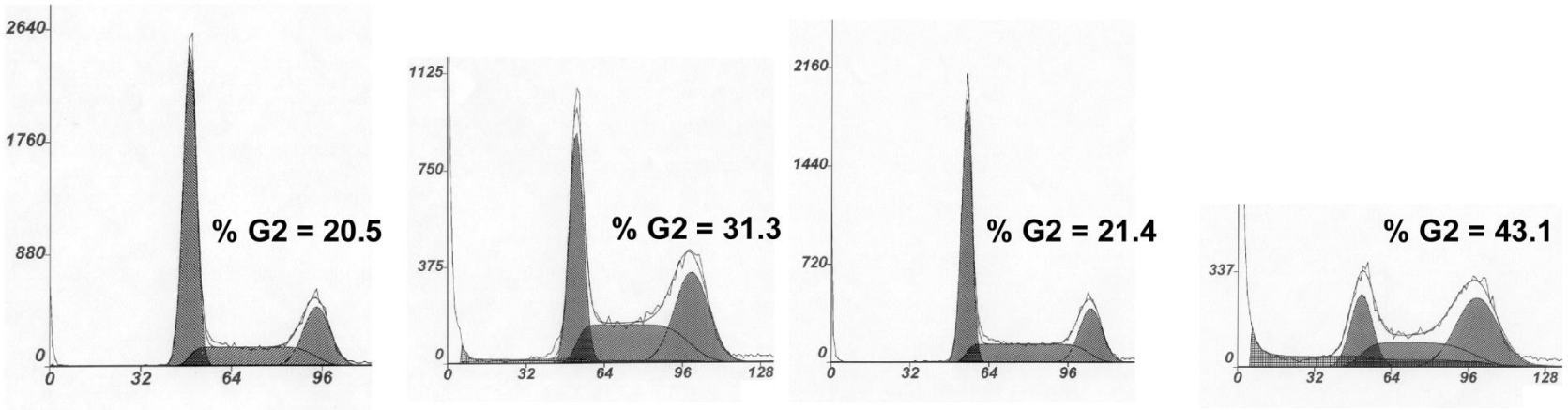
FGFR DN blocks cells at G2/M

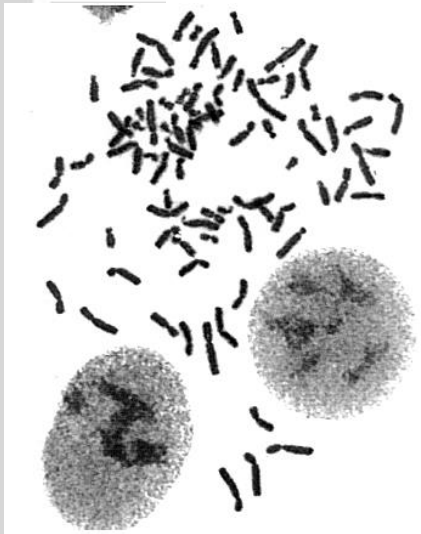
A: LNCaP

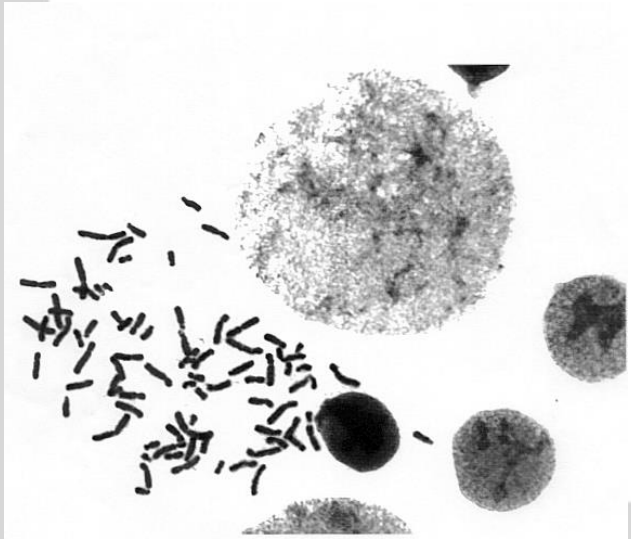


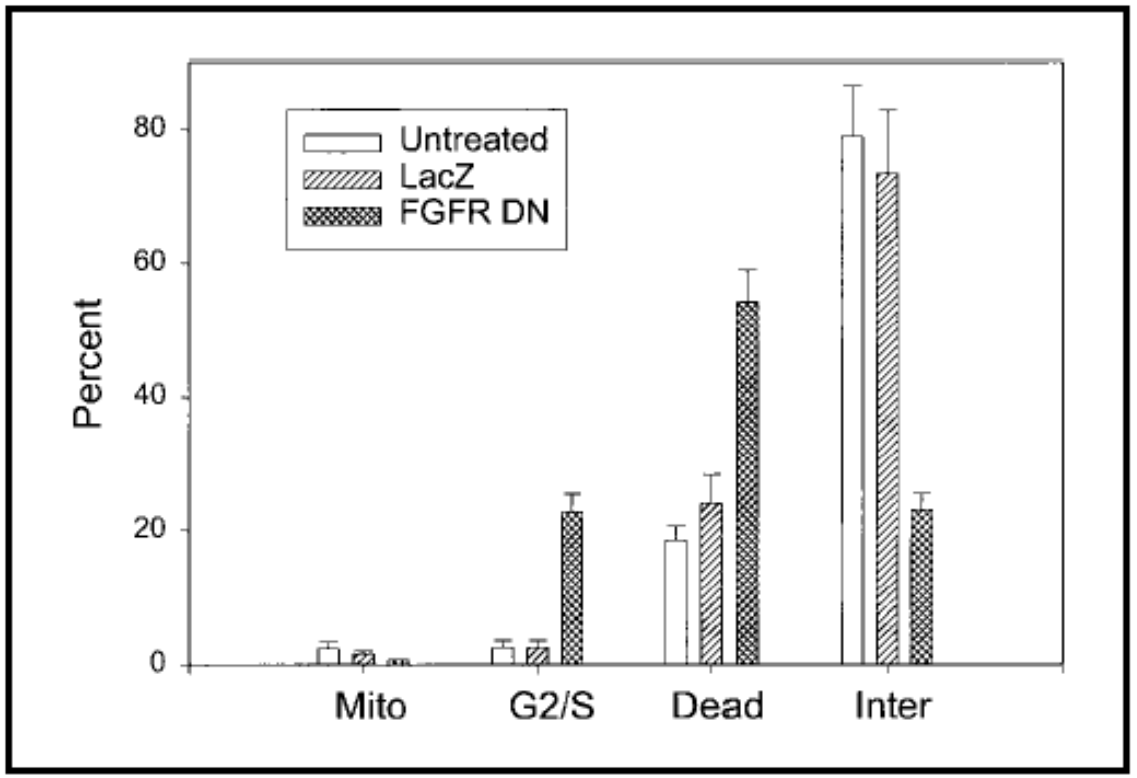
B: DU145



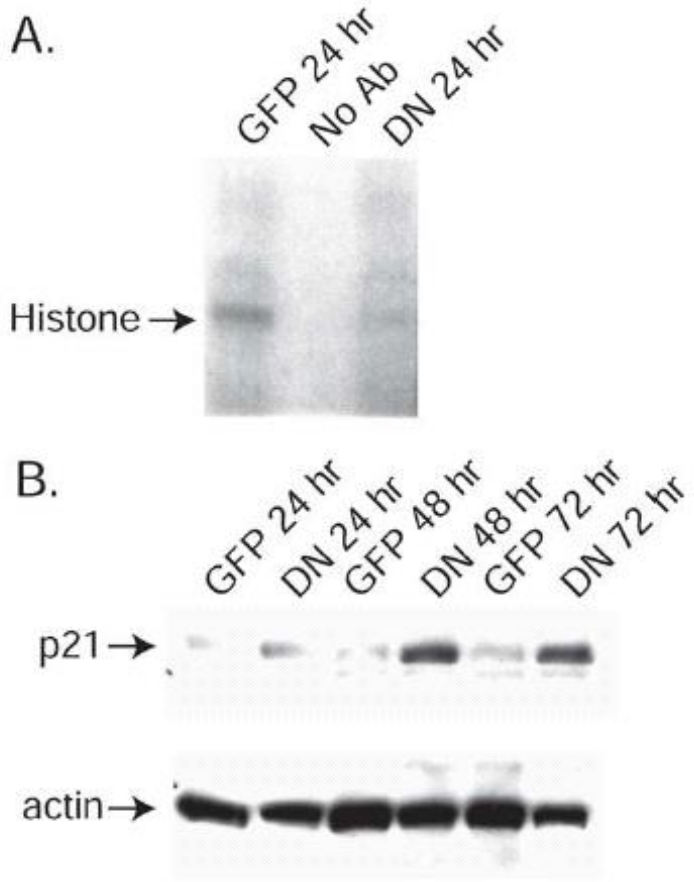
FGFR DN treated cells showed more cells at G2

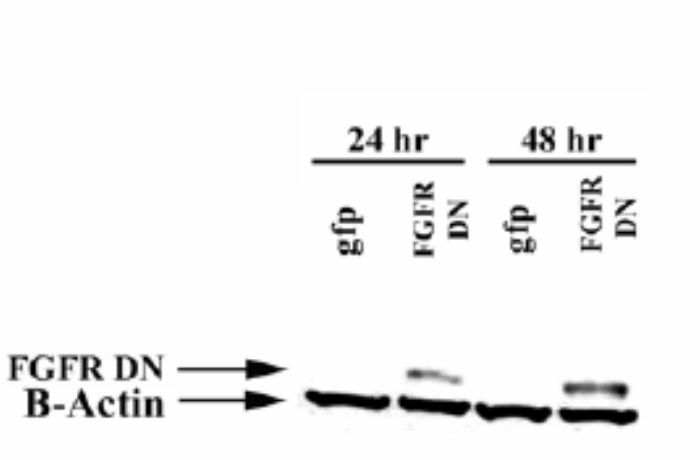


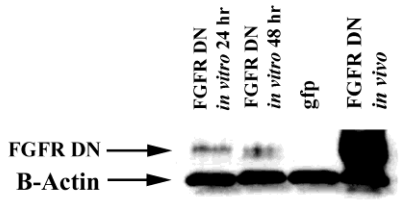




FGFR DN decrease cdc2 kinase activity and increase p21 expression




24 hr
48 hr
gfp
FGFR DN
gfp
FGFR DN
FGFR DN
B-Actin


FGFR DN in vitro 24 hr
FGFR DN in vitro 48 hr
gfp
FGFR DN in vivo
FGFR DN
B-Actin

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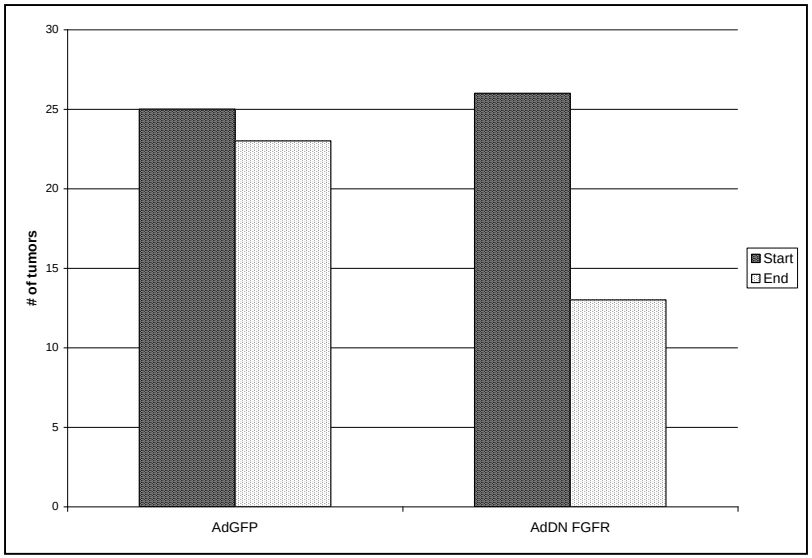
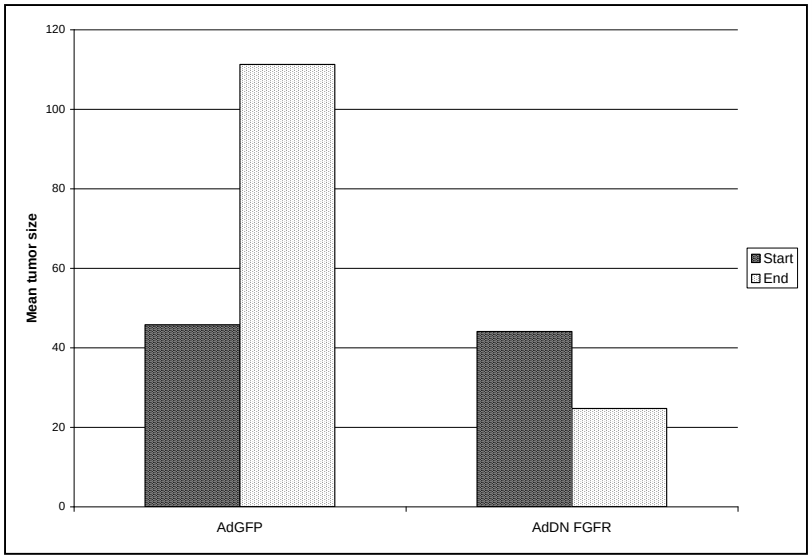
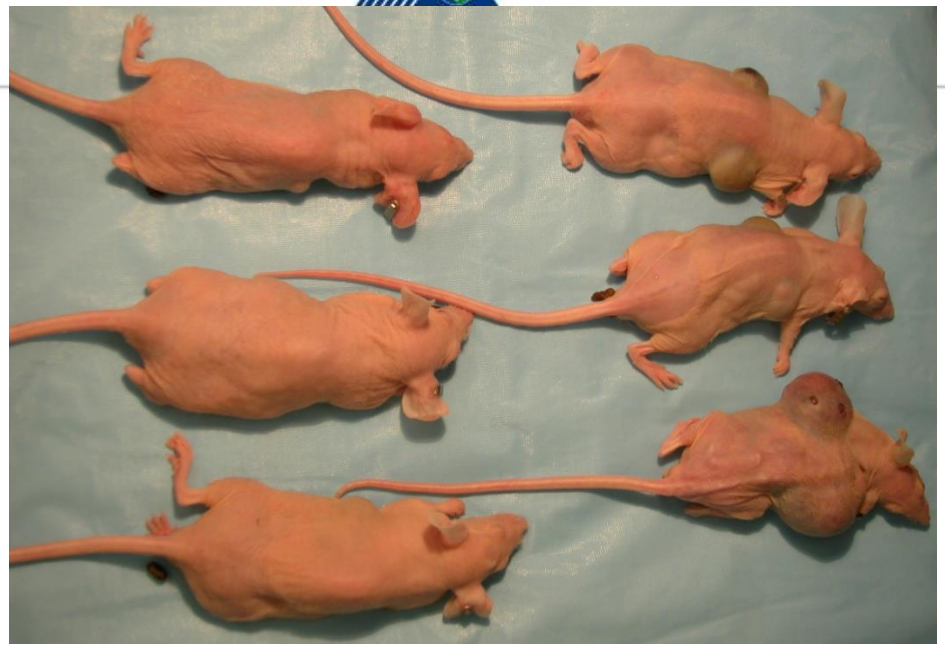
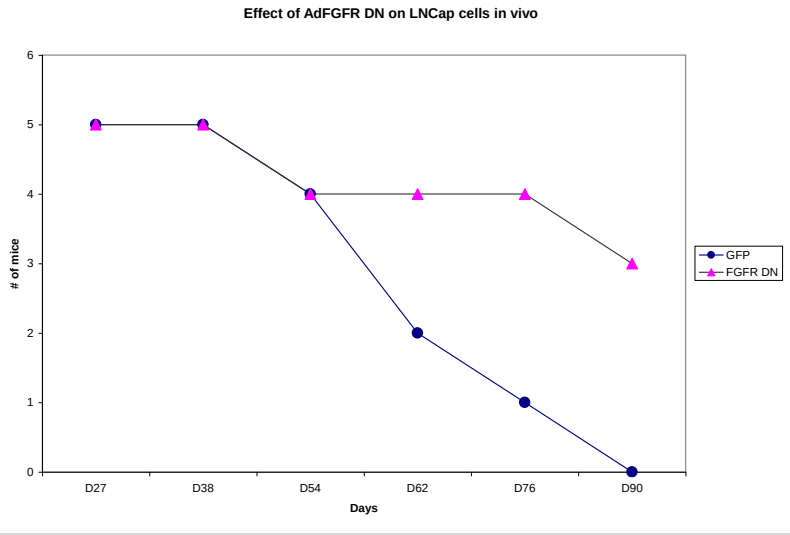
Treatment of prostate cancer xenografts with AdDN FGFR

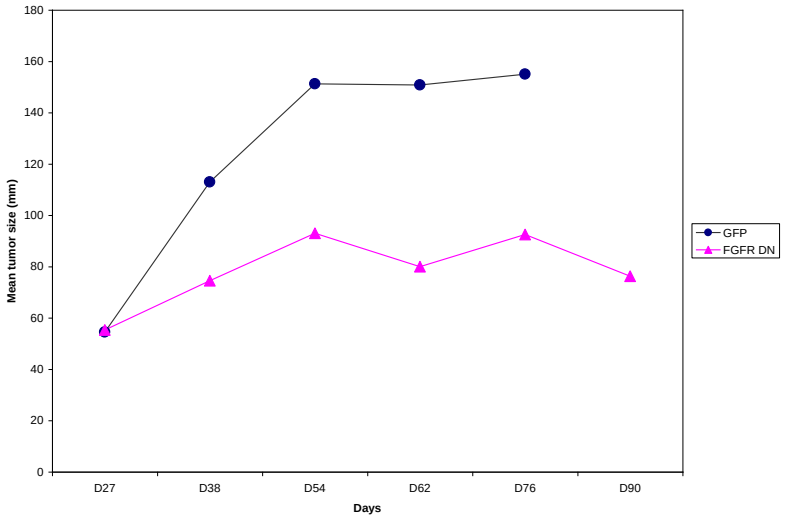


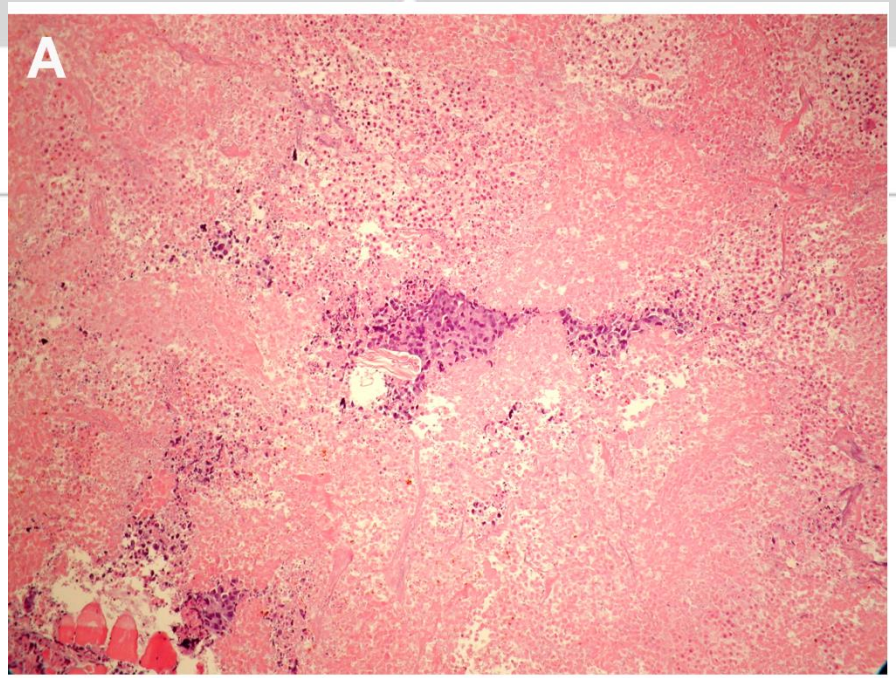


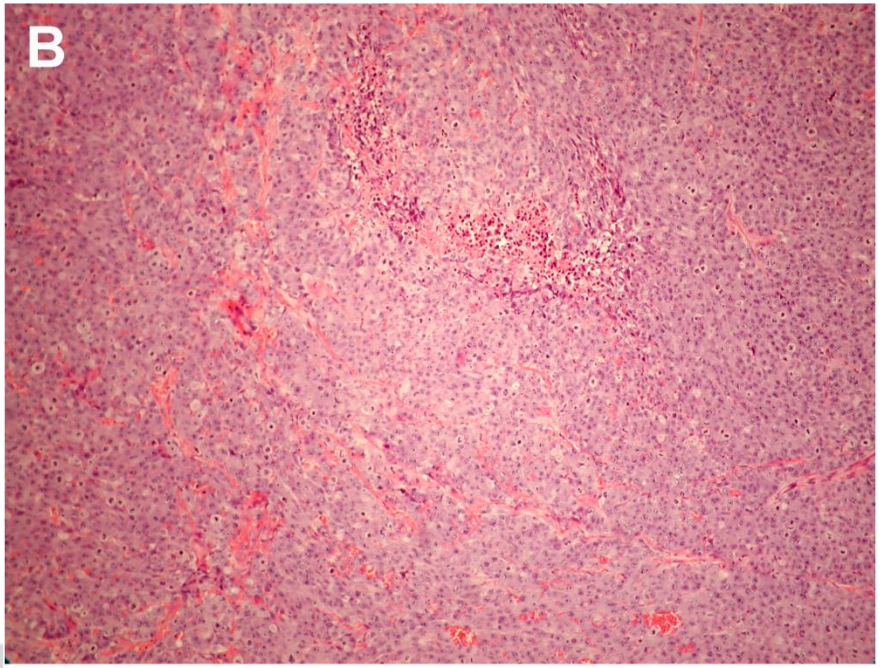
AdDN FGFR AdGFP Control



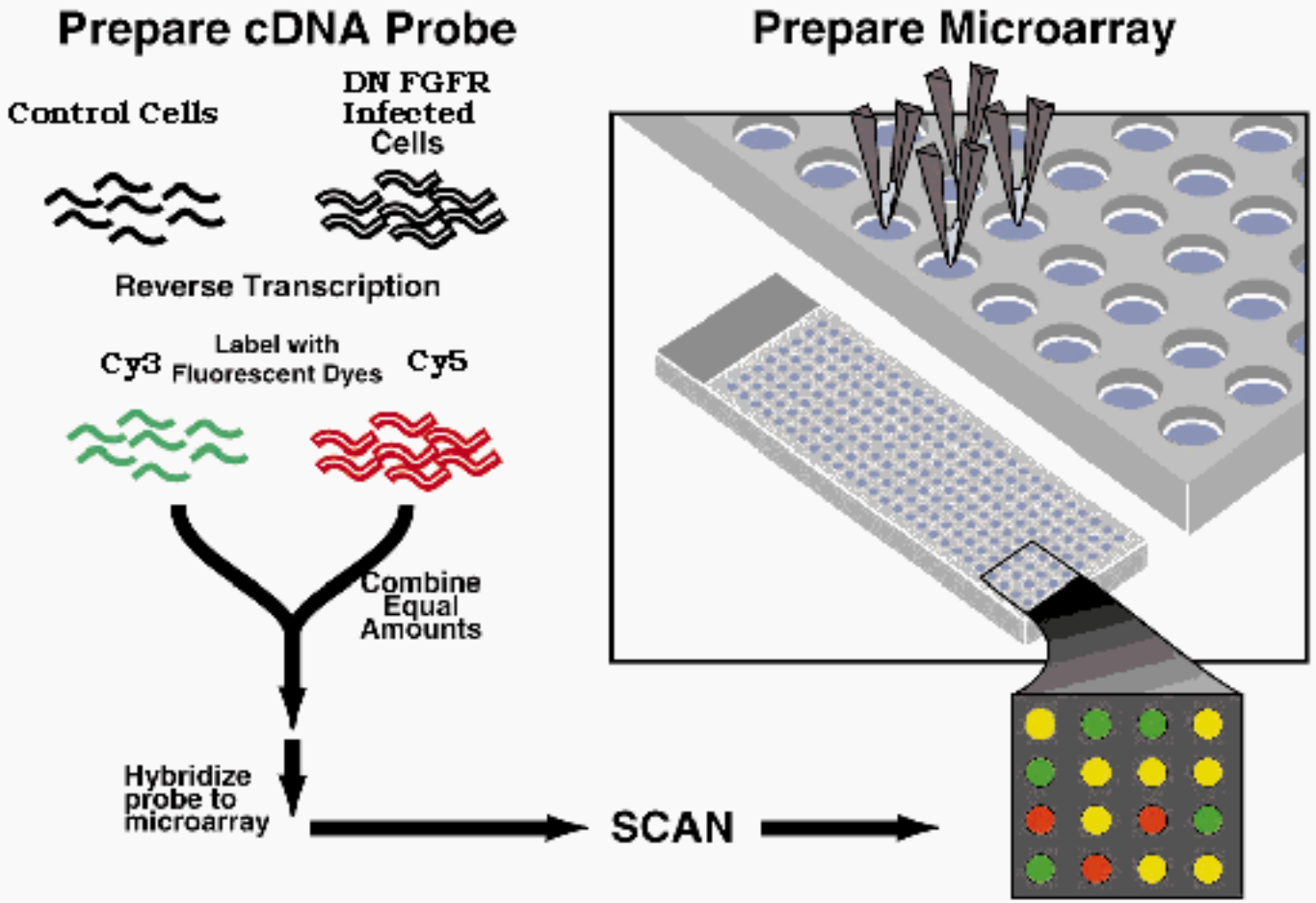





A


B

Micro-array Experiment



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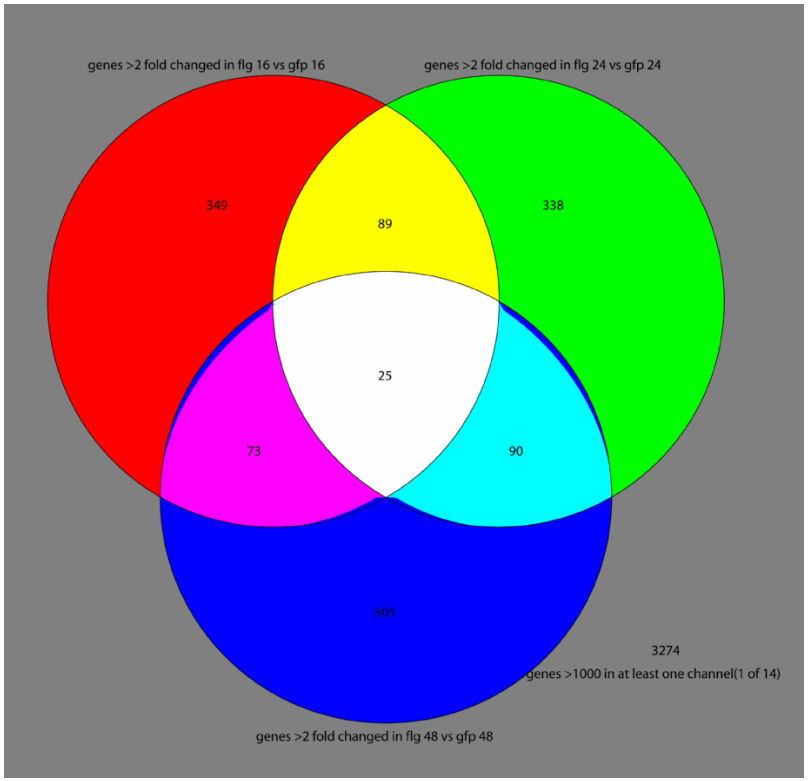
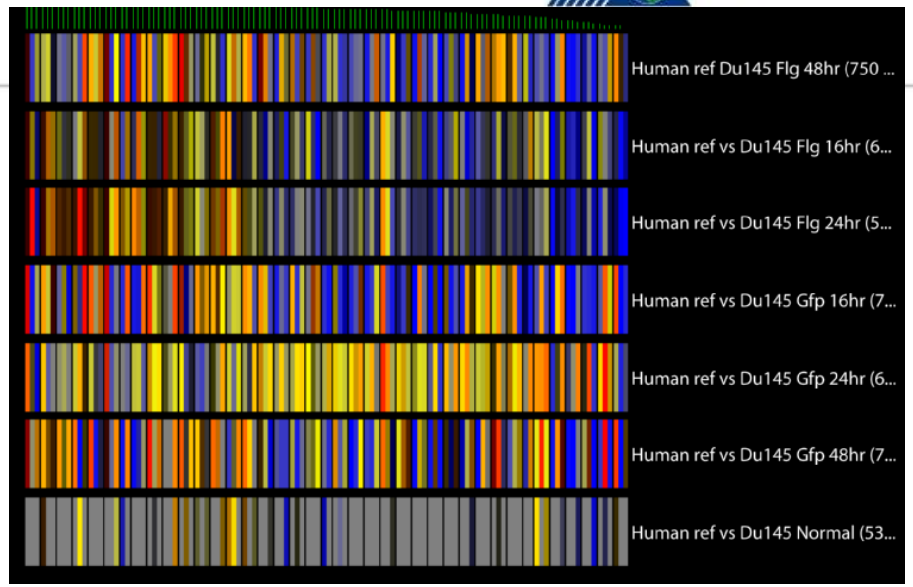
DU145

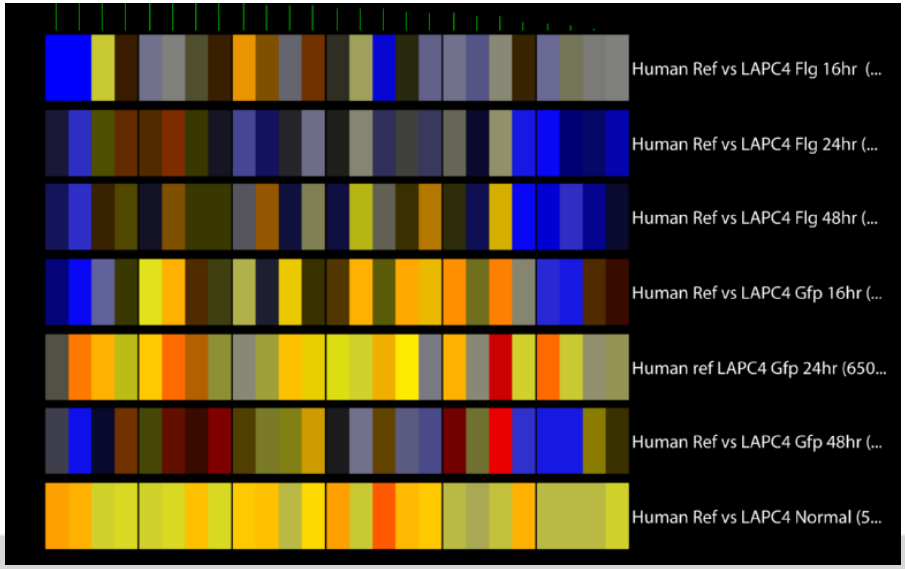
LAPC4



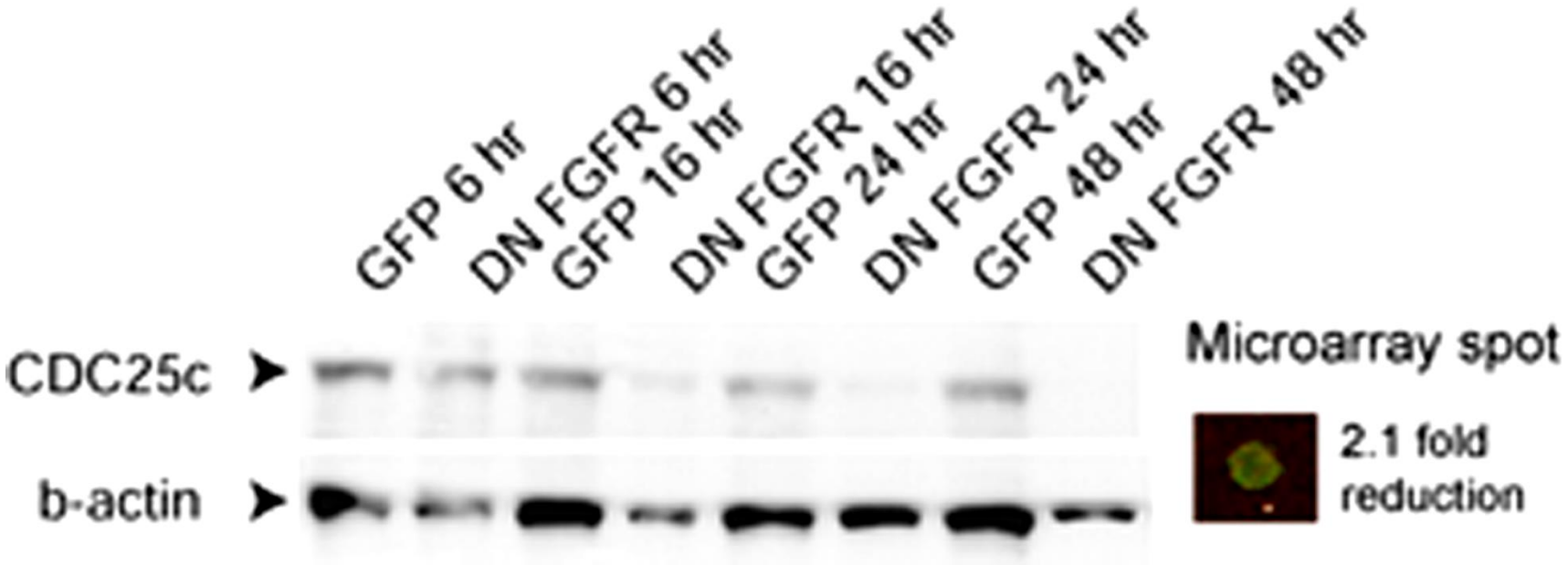
DU145


Human ref Du145 Flg 48hr (750 ...
Human ref vs Du145 Flg 16hr (6...
Human ref vs Du145 Flg 24hr (5...
Human ref vs Du145 Gfp 16hr (7...
Human ref vs Du145 Gfp 24hr (6...
Human ref vs Du145 Gfp 48hr (7...
Human ref vs Du145 Normal (53...

LAPC4


Human Ref vs LAPC4 Flg 16hr (...
Human Ref vs LAPC4 Flg 24hr (...
Human Ref vs LAPC4 Flg 48hr (...
Human Ref vs LAPC4 Gfp 16hr (...
Human ref LAPC4 Gfp 24hr (650...
Human Ref vs LAPC4 Gfp 48hr (...
Human Ref vs LAPC4 Normal (5...

Hs.65029	0.522	growth-arrest-specific protein
Hs.248	0.518	cot proto-oncogene
Hs.153752	0.516	CDC25B; CDC25HU2; M-phase induc
Hs.155324	0.507	matrix metalloproteinase 11 (MM
Hs.251674	0.499	rho6 protein
Hs.77432	0.498	epidermal growth factor recepto
Hs.30942	0.498	ephrin-B2 precursor; EPH-relate
Hs.106283	0.496	insulin-like growth factor bind
Hs.41688	0.496	dual-specificity protein phosph
Hs.59106	0.494	p53-dependent cell growth regul
Hs.77597	0.494	serine/threonine-protein kinase
Hs.656	0.491	CDC25C; M-phase inducer phospha
Hs.75379	0.491	high-affinity glutamate transpo
Hs.8724	0.491	NDR protein kinase
Hs.73986	0.489	CDC-like kinase 2 (CLK2)
Hs.101448	0.481	metastasis-associated protein 1
Hs.251674	0.479	cyclin-dependent kinase 5 activ
Hs.166681	0.465	ets-related protein tel; ets tr
Hs.79347	0.463	epidermal growth factor recepto
Hs.79126	0.463	guanine nucleotide-binding prot
Hs.173664	0.458	ERBB2 receptor protein-tyrosine
Hs.107169	0.453	insulin-like growth factor bind
Hs.79078	0.451	mitotic feedback control protei
Hs.81248	0.436	Wilms' tumor protein (WT33; WT1
Hs.75862	0.427	mothers against dpp homolog 4 (
Hs.75770	0.424	retinoblastoma-associated prote
Hs.103042	0.422	microtubule-associated protein
Hs.180911	0.416	Homo sapiens ribosomal protein


GFP 6 hr
DN FGFR 6 hr
GFP 16 hr
DN FGFR 16 hr
GFP 24 hr
DN FGFR 24 hr
GFP 48 hr
DN FGFR 48 hr
CDC25c
b-actin
Microarray spot
2.1 fold
reduction

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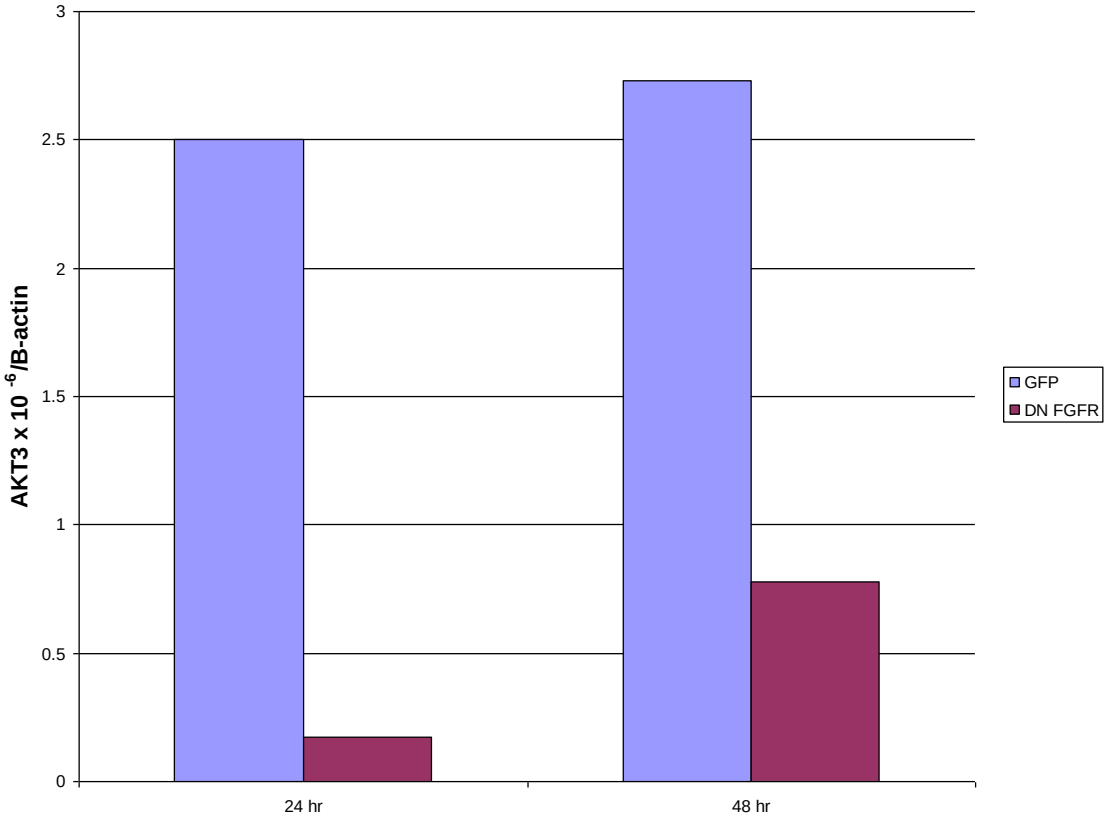
FGFR DN down-regulates AKT3



CONCLUSIONS

- **FGFs are important survival factors for prostate cancer cells.**
- **AdDN FGFR inhibits growth of human prostate cancer cell lines LNCaP and DU-145 *in vitro* and LNCaP *in vivo* as tested.**
- **This inhibition is due to the cell cycle arrest at G2 check point. Degradation of cdc25C and up-regulation of p21 molecules in DN FGFR infected cells might contribute to this inhibition.**

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ACKNOWLEDGEMENTS

- Michael Ittmann, M.D., Ph.D.
- Marco Marcelli, M.D.
- Leif Peterson, Ph.D.
- Elizabeth Davis, Ph.D.
- Alan Davis, Ph.D.
- Alka Mansukhani, Ph.D., New York University
- Sen Pathak, Ph.D., UT MD Anderson Cancer Center
- Leland Chung, Ph.D., Emory University
- Rebecca Penland
- Lori Gomez
- Shantu Dixit