

**COMPARATIVE ELISA STUDY OF NF- κ B p65
and p50, ITS INHIBITOR I κ B, AND UPSTREAM
EFFECTOR PROTEIN KINASE Akt1
EXPRESSION AND ACTIVITY IN THE TUMORS
OF BREAST CANCER PATIENTS**

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- **NF- κ B** constitutes a family of transcription factors consisting of homo- and heterodimeric complexes of proteins with common **DNA-binding domain**:

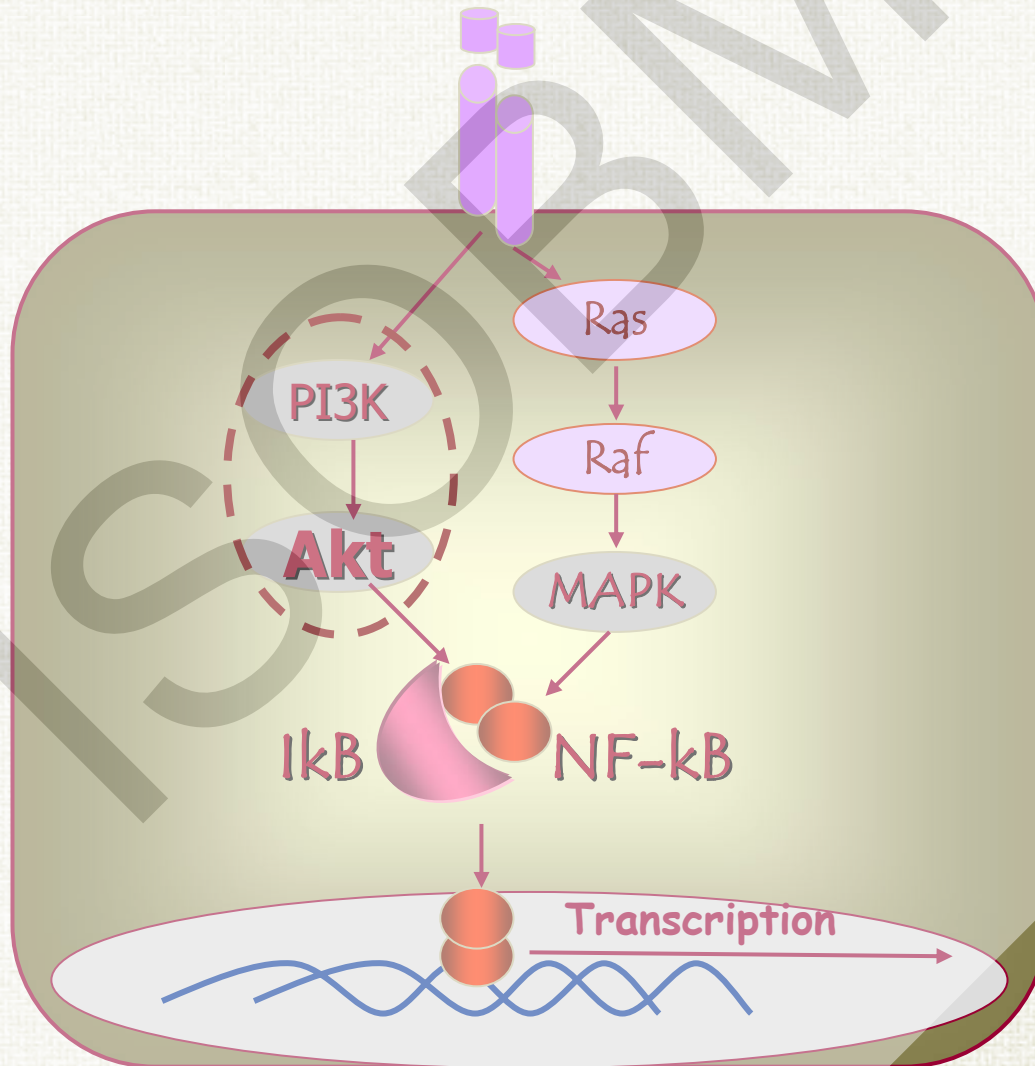
<u>Class</u>		<u>Protein</u>		<u>Aliases</u>
I	NF- κ B1	p105	→	p50
	NF- κ B2	p100	→	p52
II	RelA	p65		
	RelB			
	c-Rel			

The main form of NF- κ B in the majority of mammalian cells is **p50/RelA(p65) heterodimer**.

- **NF- κ B dimers are sequestered in the cytoplasm by I κ Bs.**

I κ B family consists of I κ B α , I κ B β , I κ B γ , I κ B ϵ , Bcl-3, p100 and p105 the best-studied and **major I κ B protein is I κ B α .**

NF- κ B activation



Preliminary results

- Increased NF- κ Bp65 and PI3K, but not phosphoAkt1 expression appears to be a widespread feature of human breast cancer.
- So, despite cooperative role of PI3K/Akt in the regulation of NF- κ B activation, tumor cell growth and survival, their involvement in clinical course and drug sensitivity of breast cancer might be quite different.

Aim of this study:

- Analysis of NF- κ B p65 and p50, its I κ B alpha inhibitor, and upstream effector protein kinase Akt1 expression and/or activation state in human breast cancer in relation to receptor status and clinico-pathologic factors.

Patients' and tumor characteristics

- Age: 23 - 4 years (median – 54).
- Premenopause - 37, perimenopause - 18, menopause - 64 patients.
- Ductal invasive cancer (DIC) – 95;
Lobular invasive cancer (LIC) – 14;
Other (mucinous, apocrine, intraductal, mixed) - 10.
- Grade I – 7; grade II – 84; grade III – 28.
- ER positive (IHC) – 70%;
PgR positive – 63%:
ER+PgR+ – 56%
- HER2 positive (**IHC 3+**) – **16%**
(IHC 2+) – 10%

TNM	n	%
T1N0M0	20	48
T2N0M0	37	
T1N1M0	7	24
T2N1M0	21	
T1N2M0	4	28
T2N2-3M0	10	
T3N0-2M0	8	
T4N1-2M0	6	
Total	119	100

The following ELISA kits were used:

- ❖ «**NF- κ Bp65 (Total)**» (Invitrogen)

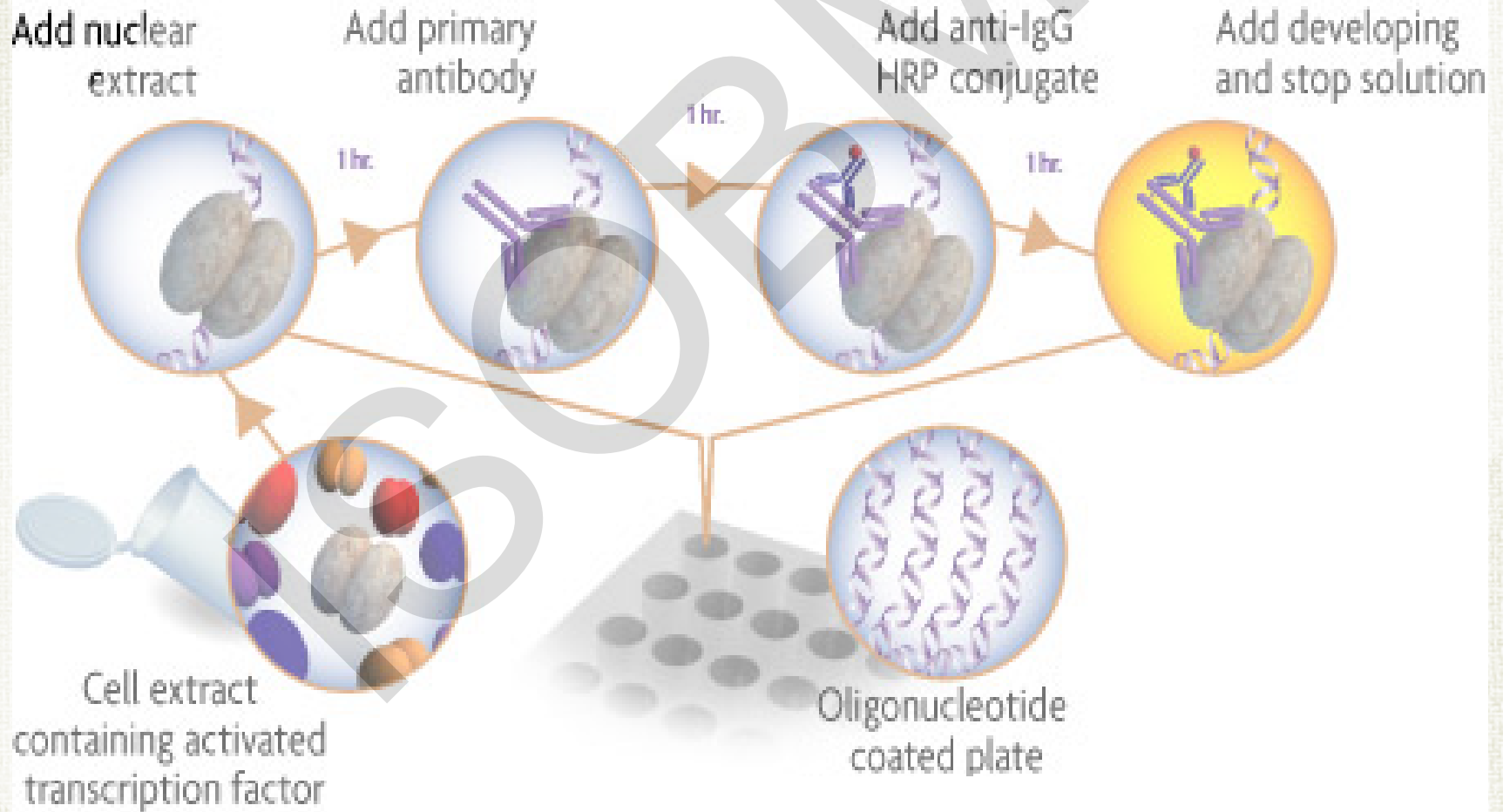
Cell Signaling Technology:

- ❖ «PathScan **Total Akt1** Sandwich ELISA Kit»,
- ❖ «PathScan **Phospho-Akt1 (Ser473)** Sandwich ELISA Kit»
- ❖ «PathScan **Total I κ Balpha** Sandwich ELISA Kit»
- ❖ «PathScan **Phospho-I κ Balpha (Ser32)** Sandwich ELISA Kit»

Active Motif:

- ❖ «TransAM™ **NF κ B p65**»
- ❖ «TransAM™ **NF κ B p50**»

TransAM™ NF-κBp65/p50 Transcription Factor Assay Kits (Active Motif, USA)

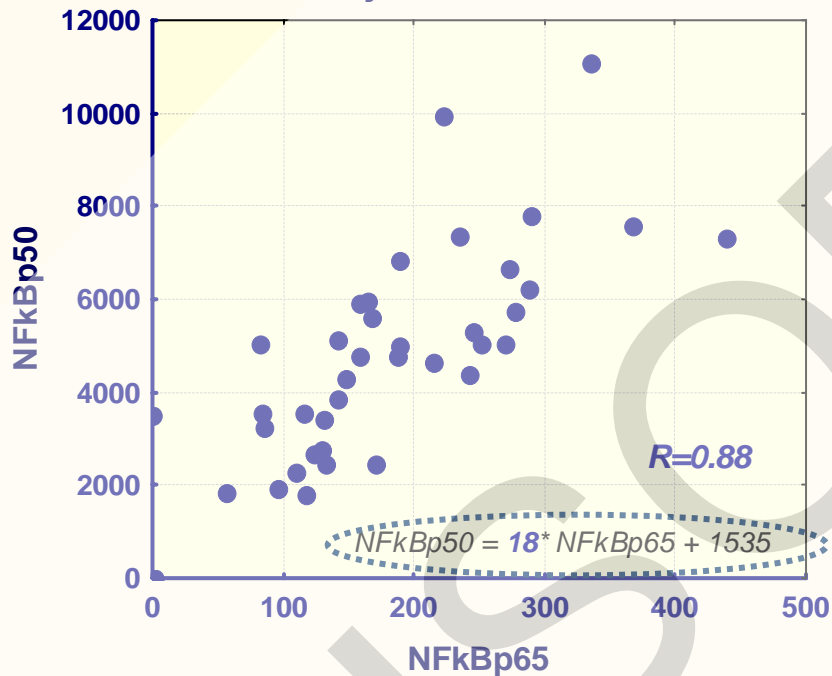


NF κ B, its inhibitors and activators in breast cancer and adjacent mammary gland tissue lysates.

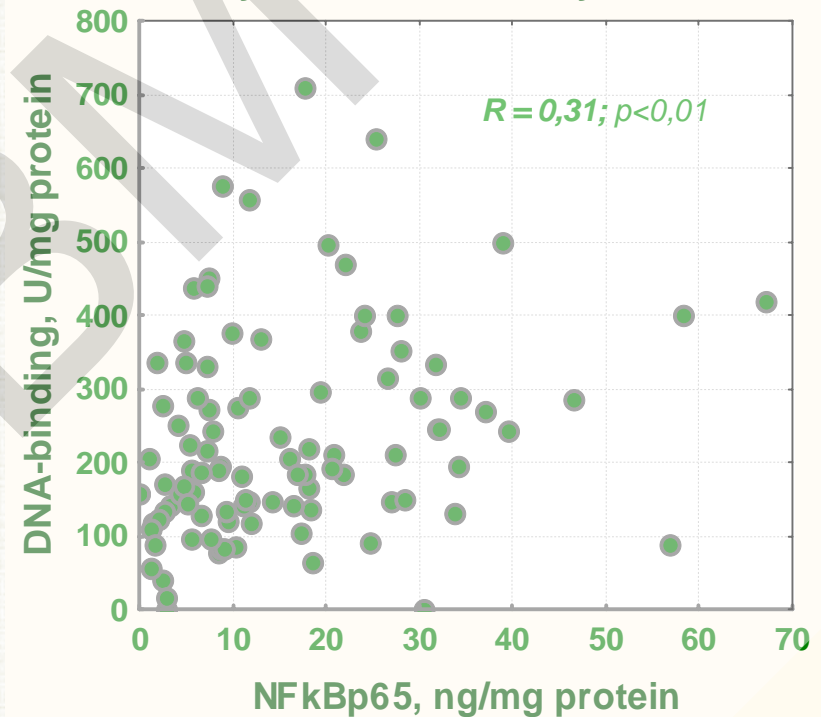
Marker	N Pts	Tumor (T)	Adjacent mammary gland (N)	T>N
Total NF-κBp65	119	11,1 0 – 67,2	2,9 0 – 42,5	86%
NF κ Bp65 DNA-binding	99	193 0 – 711	28.7 0 – 143	97%
NF κ Bp50 DNA-binding	39	479 0 – 1110	72.9 2.5 – 278	97%
Total IκBα	43	35.1 1.83 – 61.8	5.85 0 – 42.9	100%
Phospho-I κ B α (Ser32)	43	0.95 0 – 5.13	1.22 1.55 – 7.0	23%
Total Akt1	43	42.8 2.6 – 82.5	16.1 2.77 – 67.4	98%
Phospho-Akt1 (Ser473)	43	6.0 0 – 57.0	8.8 0 – 48.5	37%

General associations

NFκBp65 and NFκBp50 DNA-binding activities (U/mg protein) in breast cancer lysates



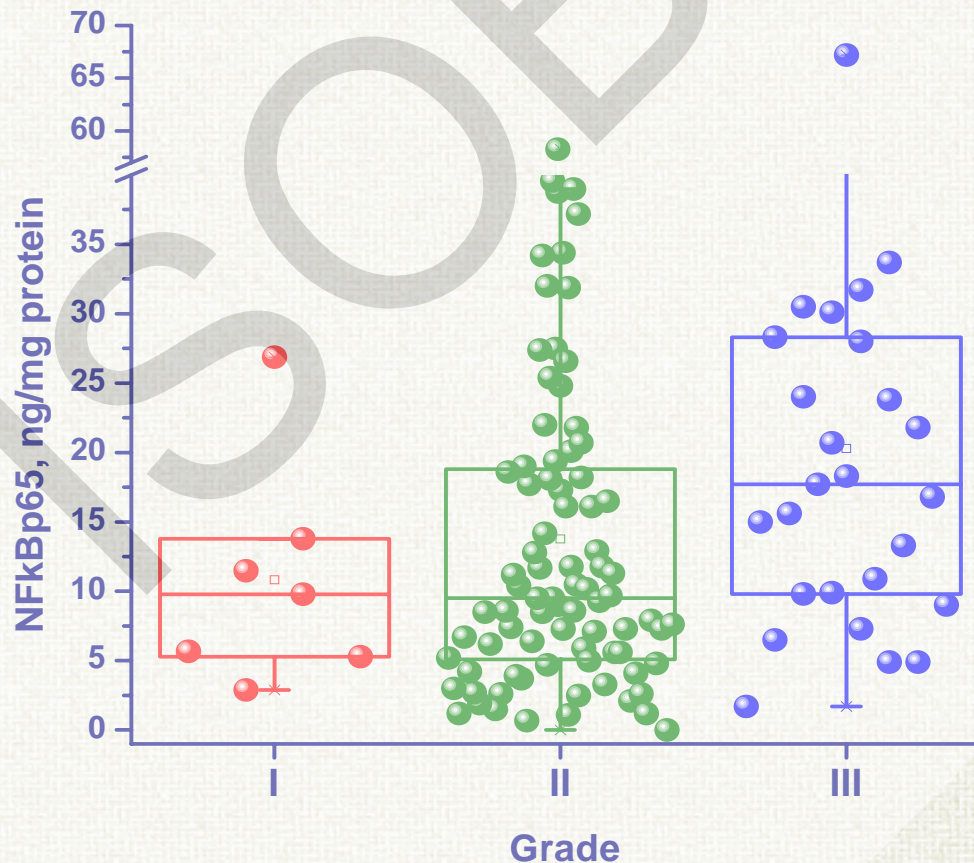
NFκBp65 protein content and DNA-binding activity in breast cancer lysates



- Tumor **NFκBp65** and **NFκBp50** DNA-binding activities were positively associated with total protein content of:
 - IκBα** - **R=0.61** ($p < 0.0001$) and **R=0.60** ($p < 0.0001$)
 - Akt1** - **R=0.37** ($p < 0.05$) and **R=0.34** ($p < 0.05$)
- **Total and Phospho Akt1** levels were positively associated: **R=0,38** ($p < 0,05$)
- **NF-κBp50** DNA-binding was positively associated with **phospho-Akt1**: **R=0.34** ($p < 0.05$)

Clinico-pathologic analysis






- **No** significant associations were found between the majority of parameters studied and **disease stage**, tumor size (**T**) and **histologic type**, lymph node status (**N**).
- Only **NF- κ Bp65 content increased with grade**, and in grade III tumors was significantly higher than in grade II tumors ($p < 0.05$):



NF-κBp65 content and DNA binding activity depending on breast cancer receptor status

Receptor status	N	NF-κBp65 ng/mg protein	NF-κBp65 DNA-binding activity U/mg protein	NF-κBp65 DNA-binding activity U/mg NF-κBp65
ER ⁺	81	8,95 0 – 67,2	187 0 - 640	15,7 0 – 182
ER ⁻	34	13,7 2,1 – 46,5	223 63,5 - 497	15,8 3,42 – 77,8
ER ⁺ PgR ⁺	65	9,47 0 – 58,3	193 0,98 - 640	19,3 0,34 - 182
ER ⁻ PgR ⁻	31	13,3 2,08 – 46,5	212 63,5 - 497	16,0 3,42 – 77,8
HER2 ⁺	18	18,4 4,25 – 67,2	166 63,5 – 497	9,29 3,42 – 45,3
HER2 ⁻	97	9,73 0 – 58,3	191 0 - 640	16,7 0 - 182
ER ⁺ PgR ⁺ HER2 ⁻	62	9,38 0 – 58,3	193 0,98 - 640	19,3 0,34 - 182
ER ⁺ PgR ⁺ HER2 ⁺	3	21,8 7,41 – 33,7	202 131 - 272	20,3 3,90 – 36,7
ER ⁻ PgR ⁻ HER2 ⁺	13	18,3 4,25 – 46,5	158 63,5 - 497	9,29 3,42 – 37,2
ER ⁻ PgR ⁻ HER2 ⁻	18	10,2 2,08 – 34,4	282 123 - 450	27,5 8,35 – 77,8

Conclusions

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-  In more than 90% of human breast cancers a **coordinate increase of NF- κ Bp65 and p50 DNA-binding activities** is observed. Total NF- κ Bp65, I κ B α and effector protein kinase Akt1 concentrations are also increased.
 -  **NF- κ Bp50** DNA-binding activity is several times higher than that of NF- κ Bp65 and is positively **associated with activated (phospho) Akt1**.
 -  There are **no significant associations** between these markers (except NF- κ Bp65 content) and main breast cancer clinico-pathologic features including steroid receptors status.
 -  **NF- κ Bp65** concentration in **HER2+** tumors is higher than in **HER2-**, but the reverse relation is observed for its specific DNA-binding activity.

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Thank you for your attention

