Signal Transduction of the ErbB RTK Family

Nancy Hynes
Friedrich Miescher Institute for Biomedical Research
Basel, Switzerland

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DISCLOSURE SLIDE

I have nothing to disclose

Overview of ErbB receptors

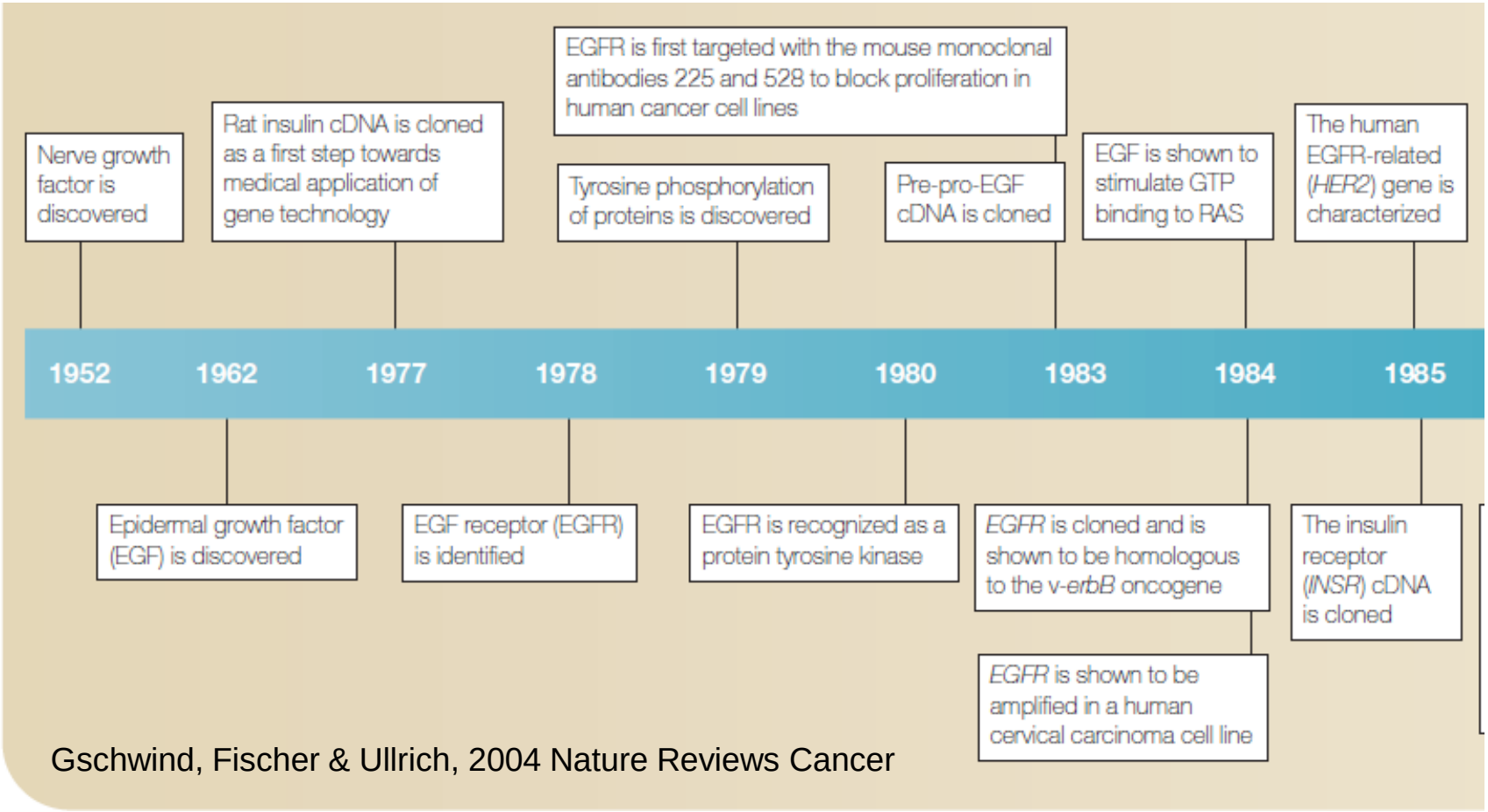
Mutations in human cancers

Activation mechanisms
Role of ErbB2
ErbB2/ErbB3 heterodimers

Novel downstream regulator of metastasis

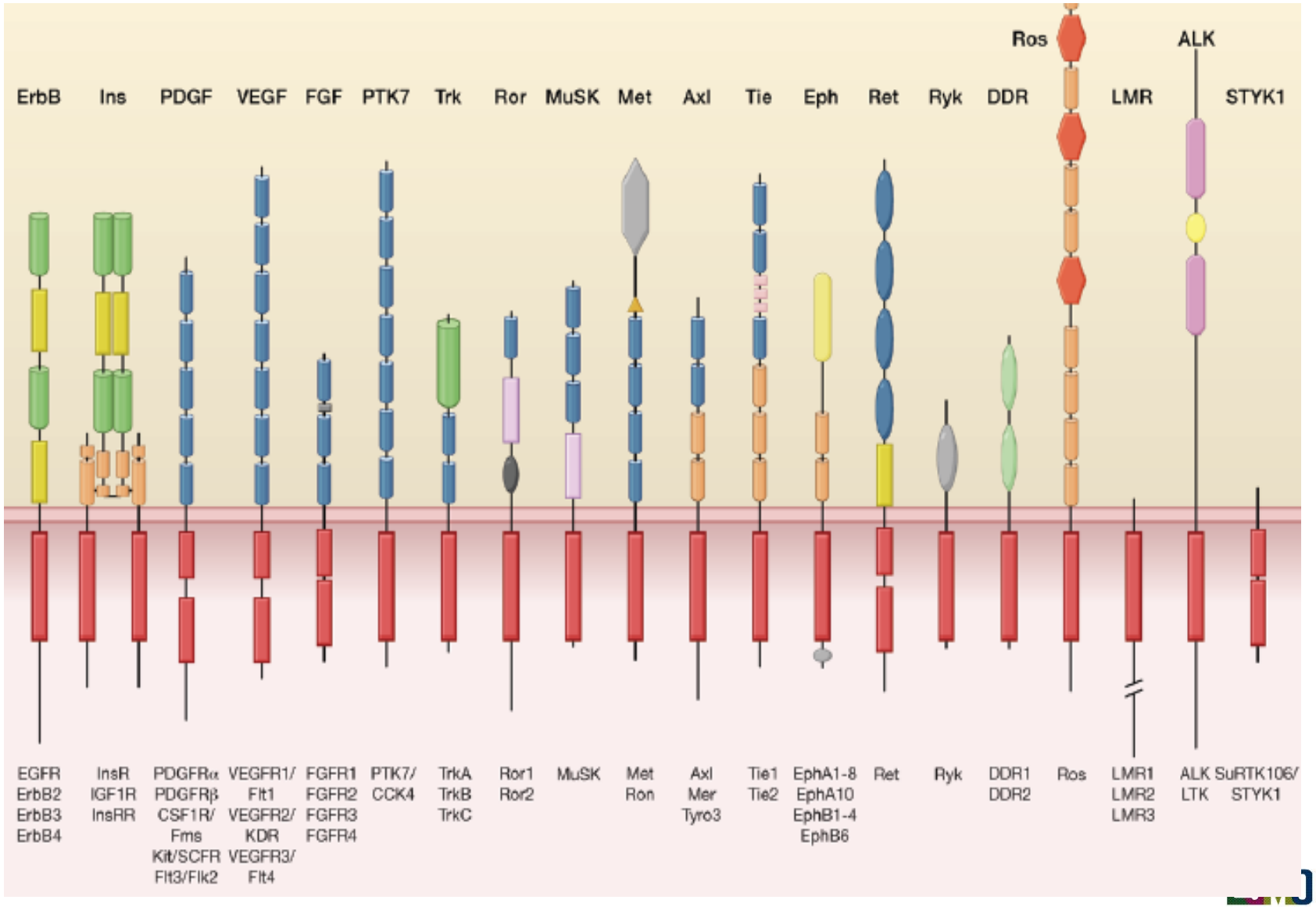
RET receptor and breast cancer

The ErbB/EGFR Receptor Tyrosine Kinase Family
the first sub-group of the RTK family

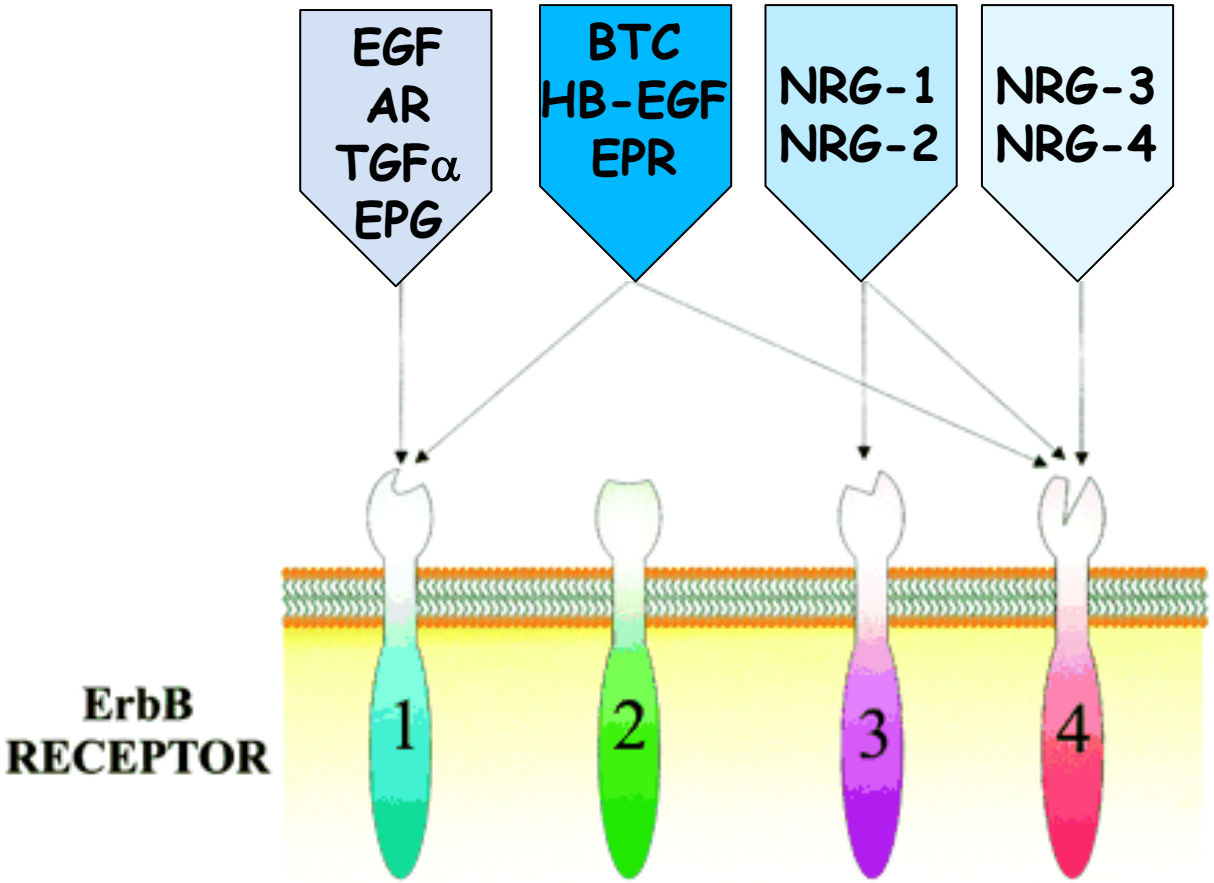


Gschwind, Fischer & Ullrich, 2004 Nature Reviews Cancer

Receptor Tyrosine Kinases:
58 members in 20 structurally diverse families



The ErbB Receptor Tyrosine Kinase / Ligand Network



EGFR
ErbB1

HER2
ErbB2
Neu

HER3
ErbB3

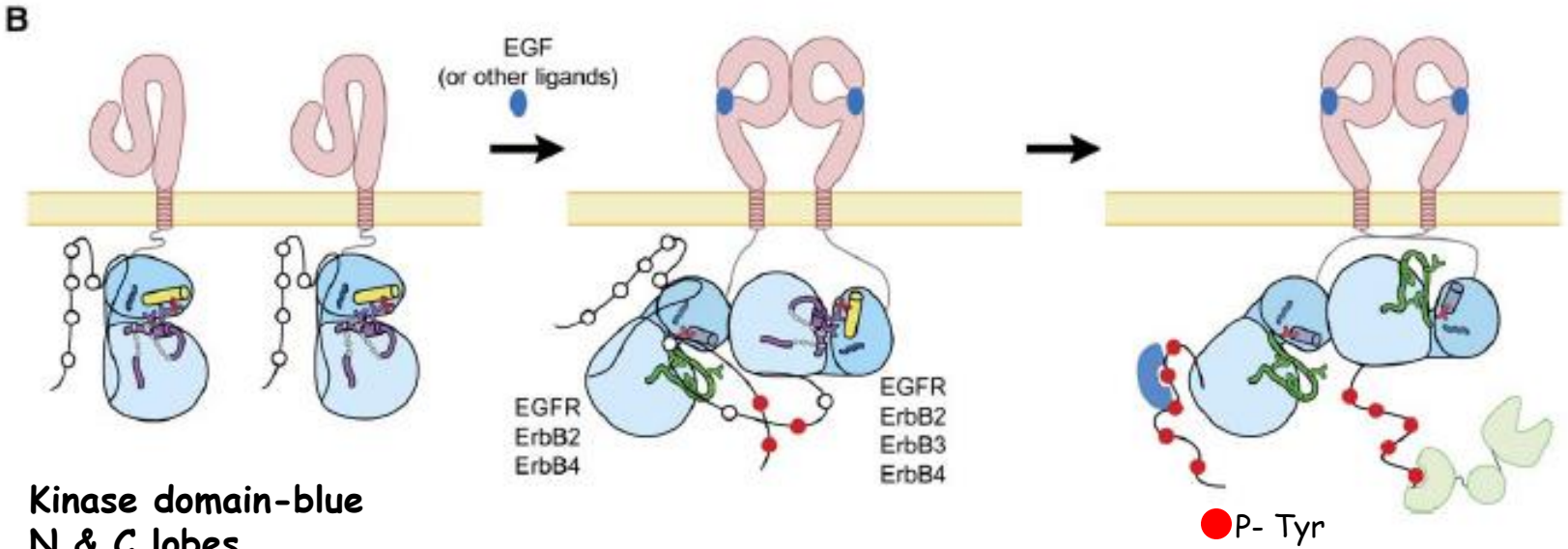
HER4
ErbB4

Activation of intracellular kinase domain

How does ligand-induced dimerization activate the intracellular tyrosine kinase domain?

- Each TKD is cis-autoinhibited by intra-molecular interactions

- Ligand induced dimerization releases the intra-molecular interactions leading to kinase activation.



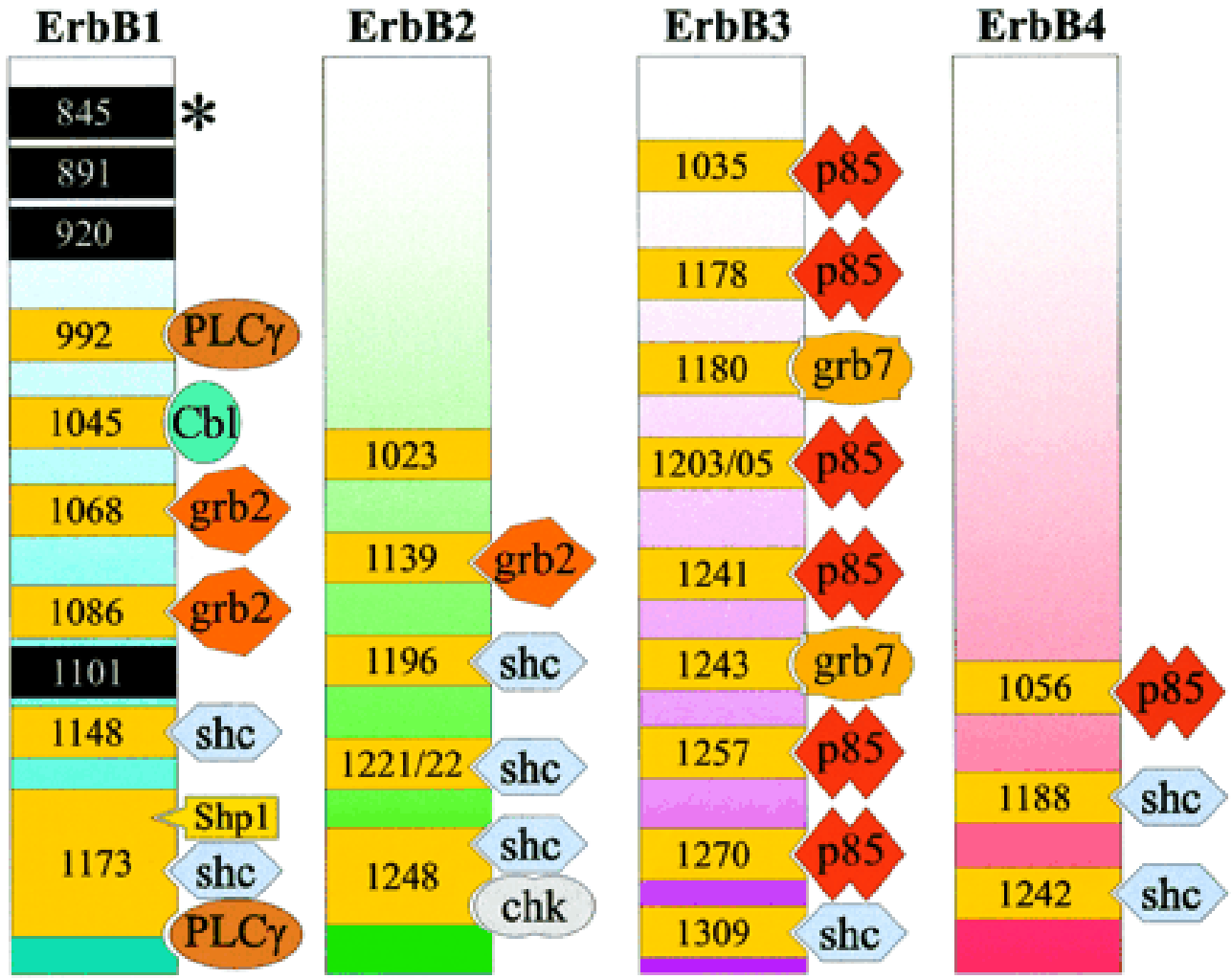
Kinase domain-blue
N & C lobes

Intracellular signaling proteins: SHC, PI3K …

Bind P-Tyr via SH2 or PTB domains

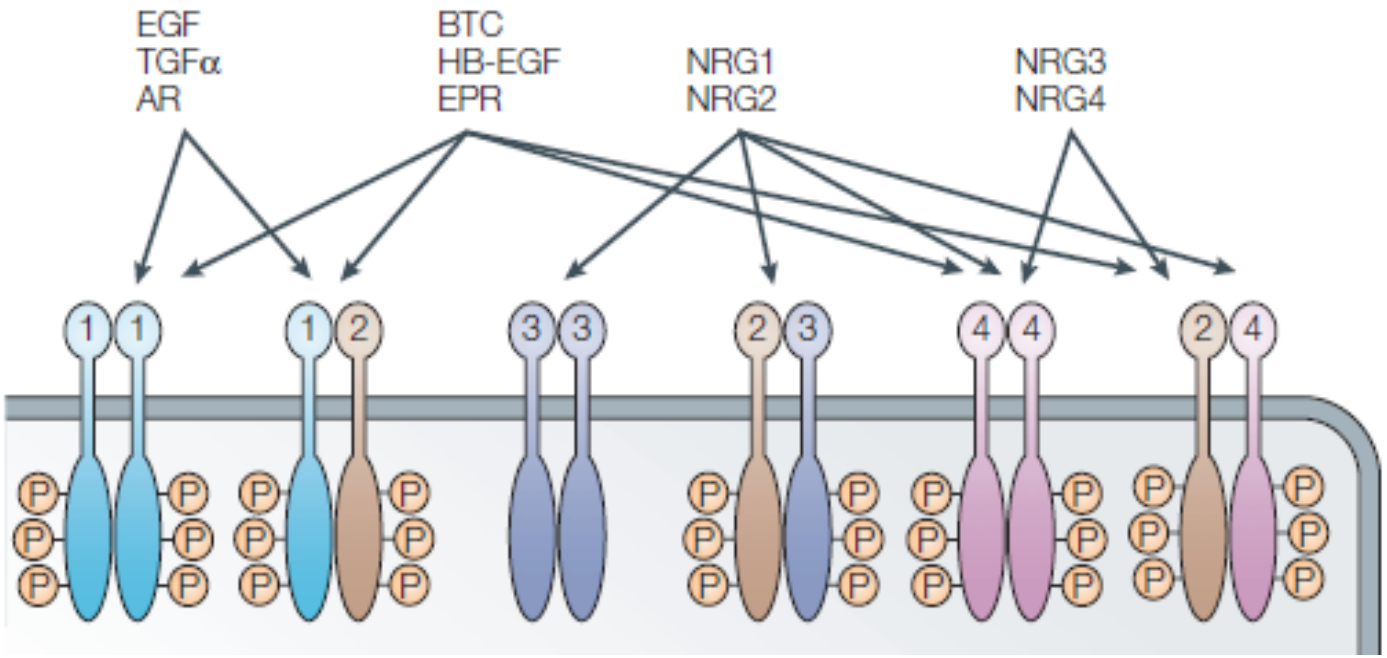
X. Zhang et al 2006 Cell

P-Tyr residues bind SH2 and PTB-domain containing proteins… that stimulate signaling pathways



-each receptor has a distinct pattern of binding proteins
-leads to signaling diversity

Ligand binding induces receptor homo and heterodimers



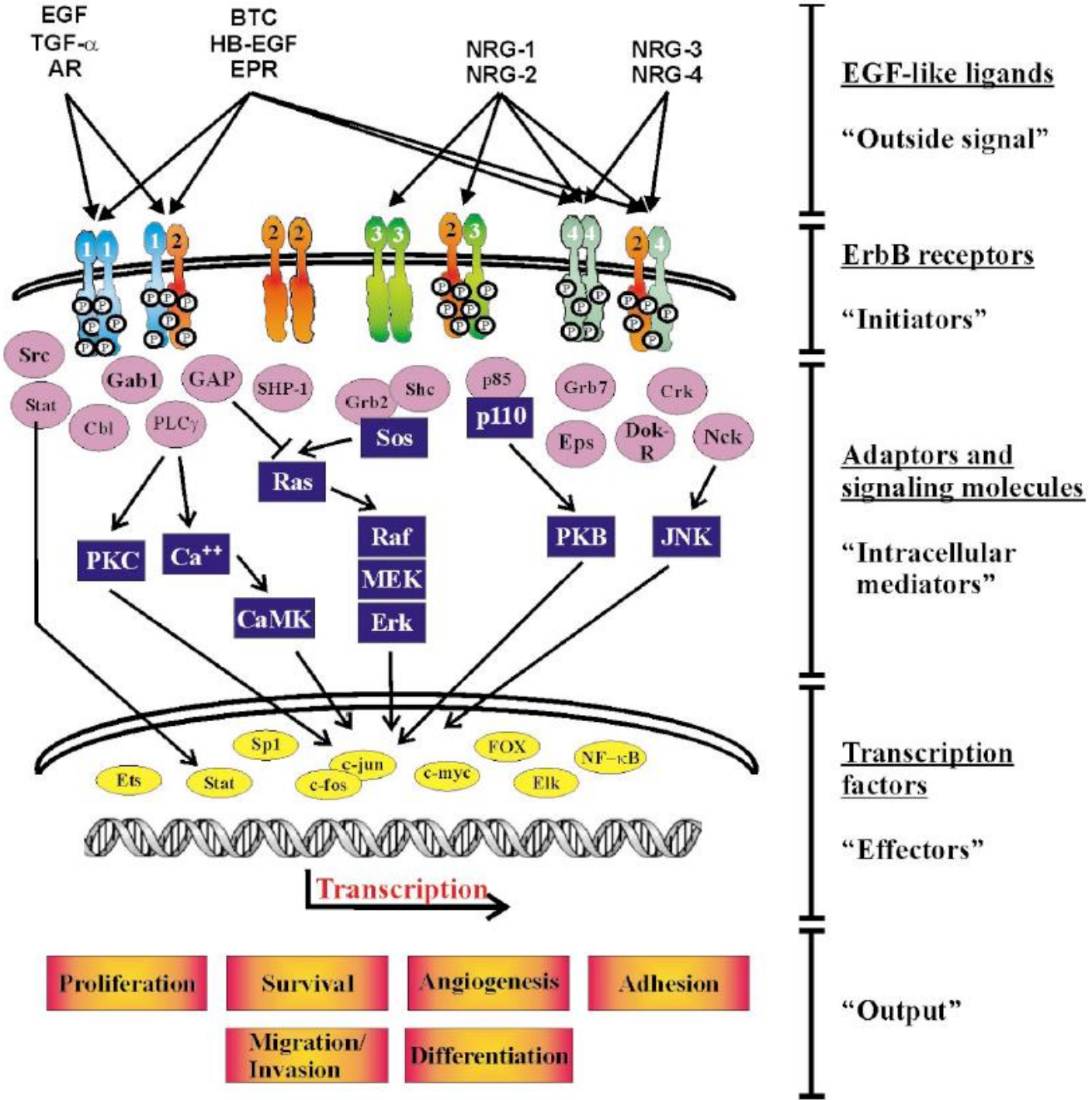
By examining cells engineered to express pairs of ErbB receptors, we found that heterodimers activate distinct signaling pathway;

….an additional mechanism to diversify signaling potential

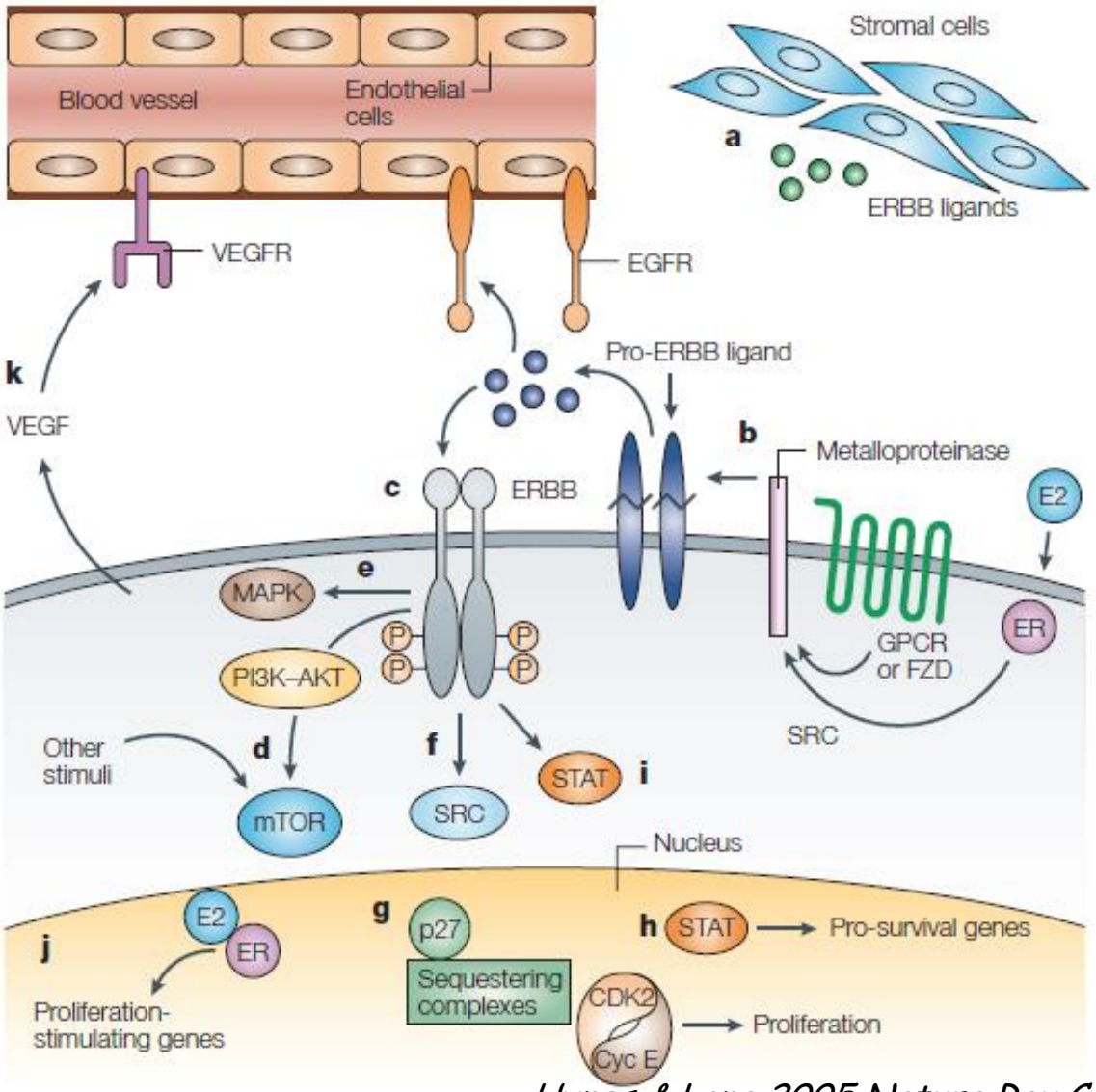
ERBB RECEPTORS: Directing Key Signaling Networks Throughout Life

Thomas Holbro and Nancy E. Hynes

Annu. Rev. Pharmacol. Toxicol. 2004. 44:195–217

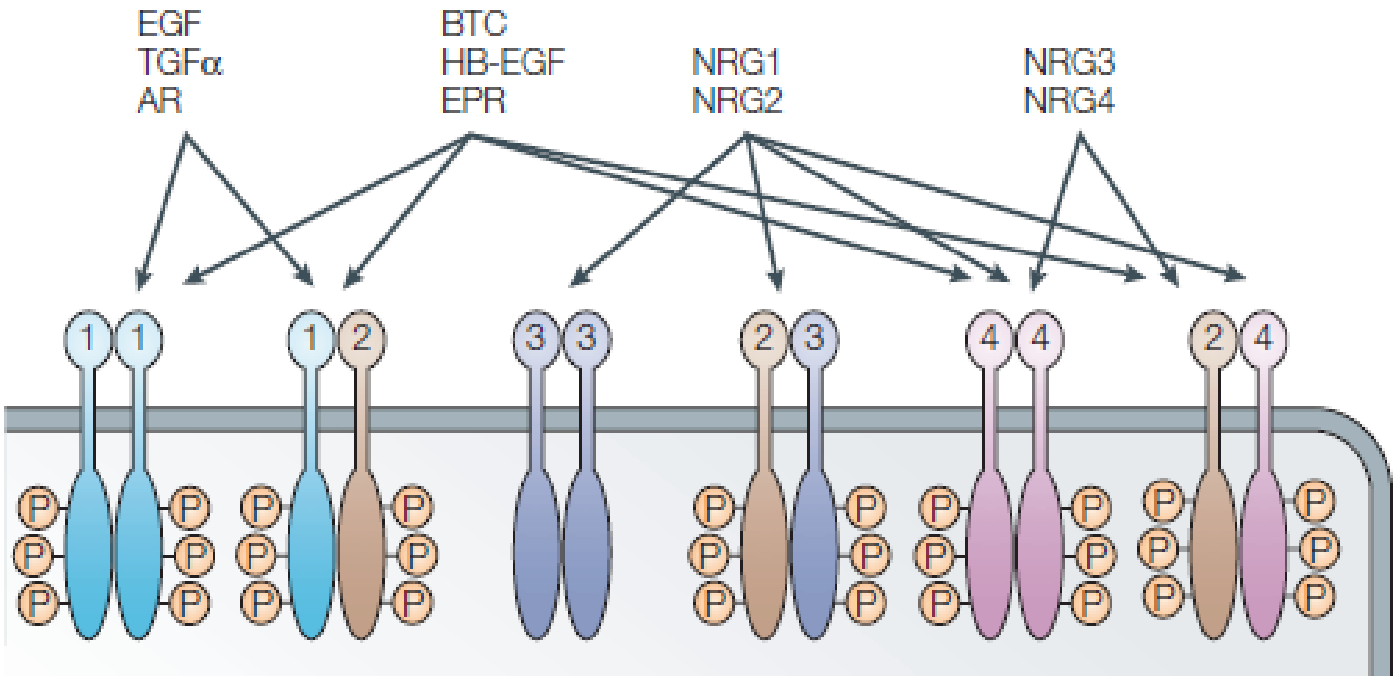


Signaling complexity increases in the context of the tumor and its environment



Hynes & Lane 2005 Nature Rev Cancer

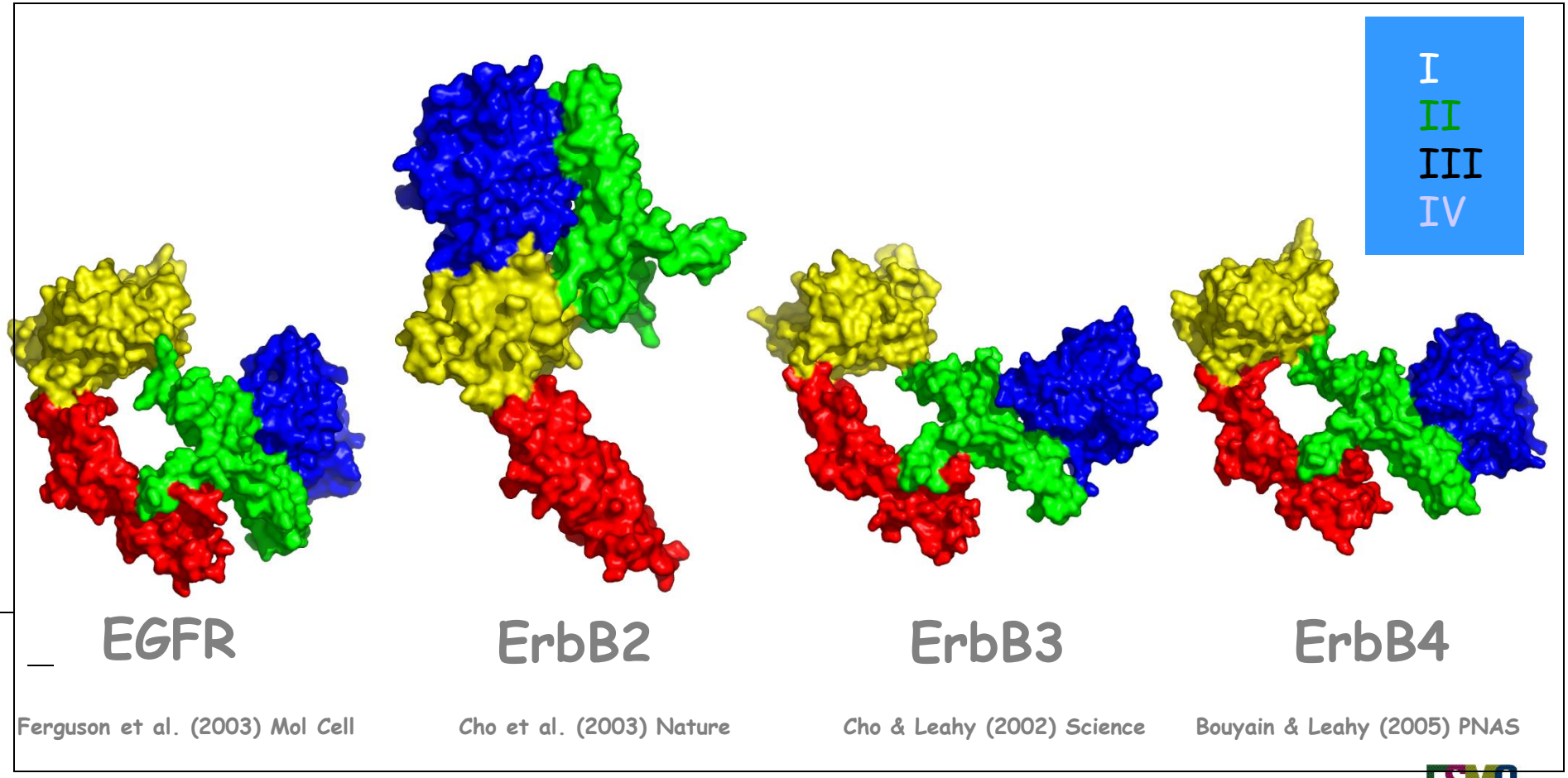
ErbB2 does not bind any of the EGF family ligands, but is activated as a heterodimer with the other ErbBs



Hynes & Lane 2005 Nature Rev Cancer

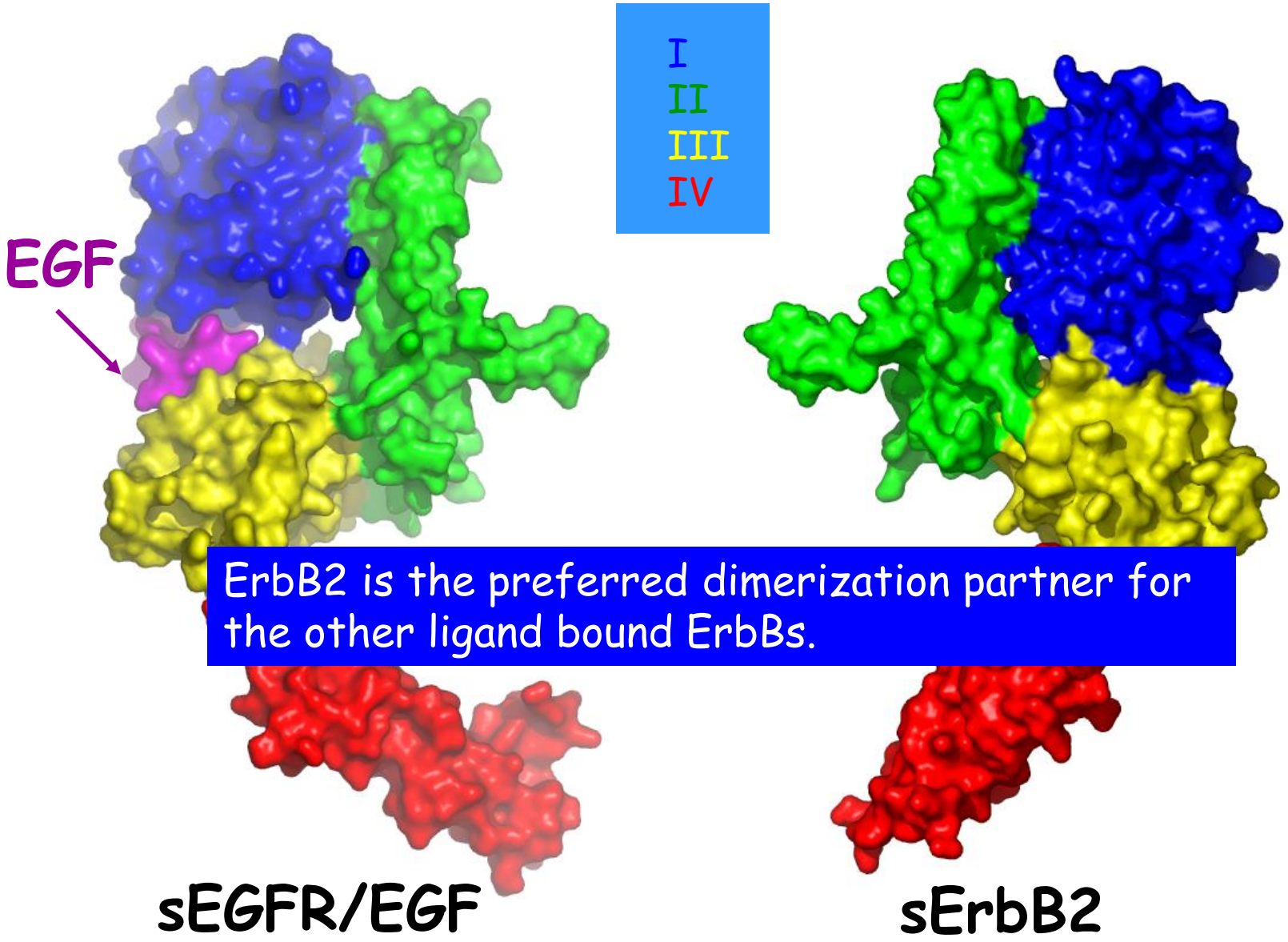
Ectodomain structures of the ErbB receptors

- In the absence of ligands, ectodomains of EGFR, ErbB3 & ErbB4 are "closed".
- ErbB2 has an extended structure.



Provided by D. Leahy

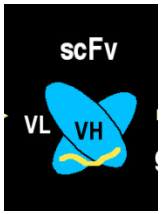
ErbB2 structure is similar to ligand-bound EGFR



THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 269, No. 39, Issue of September 30, pp. 23931–23936, 1994
Printed in U.S.A.

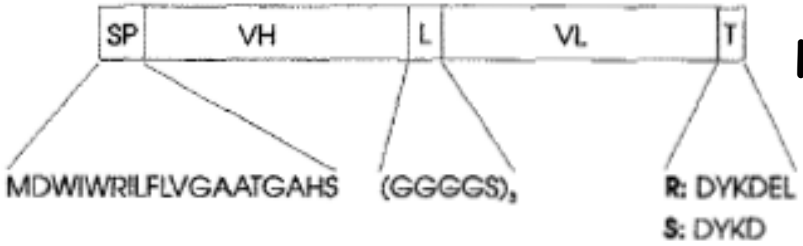
Intracellular Expression of Single Chain Antibodies Reverts ErbB-2 Transformation*

Roger R. Beerli, Winfried Wels‡, and Nancy E. Hynes§
From the Friedrich Miescher-Institut, P. O. Box 2543, CH-4002 Basel, Switzerland



ErbB2 specific scFv expressed in cells & provided with an Endoplasmic reticulum retention signal

Captures ErbB2 in the ER preventing its localization to the plasma membrane.



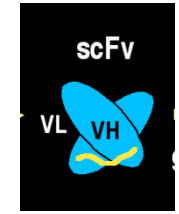
KDEL – ER retention signal

FIG. 1. **Diagram of the secreted (*S*) and ER-retained (*R*) forms of scFvs FRP5 and FWP51.** The N-terminal signal peptide (*SP*), the heavy chain variable domain (*VH*), the linker peptide (*L*), the light chain variable domain (*VL*), and the C-terminal tags (*T*) are indicated. *DYKD*: FLAG epitope; *DYKDEL*: FLAG epitope/ER retention signal.

ESMO

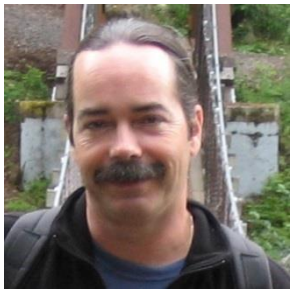
The EMBO Journal Vol.16 No.7 pp.1647–1655, 1997

ErbB-2, the preferred heterodimerization partner of all ErbB receptors, is a mediator of lateral signaling

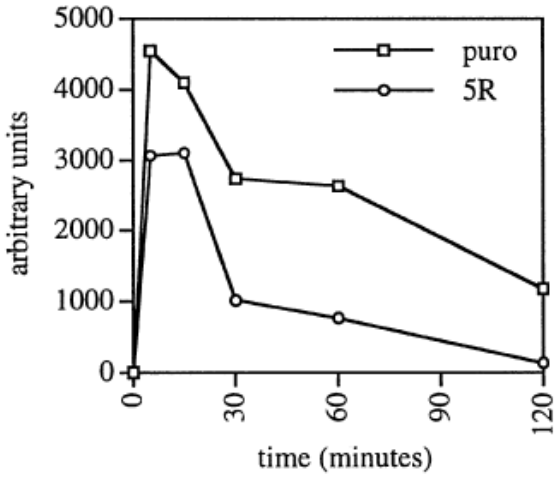


Diana Graus-Porta

Roger Beerli

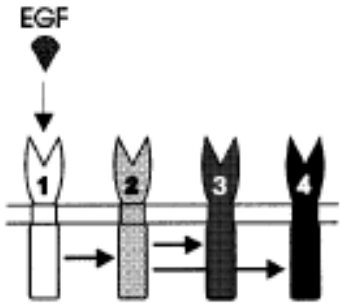


Expressed ErbB2 specific 5R-scFv to retain ErbB2 in the ER; tested for effects of ligands on signaling

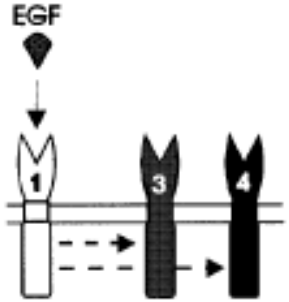


Kinetics of Erk signaling in response to an EGFR ligand

Control puro cells

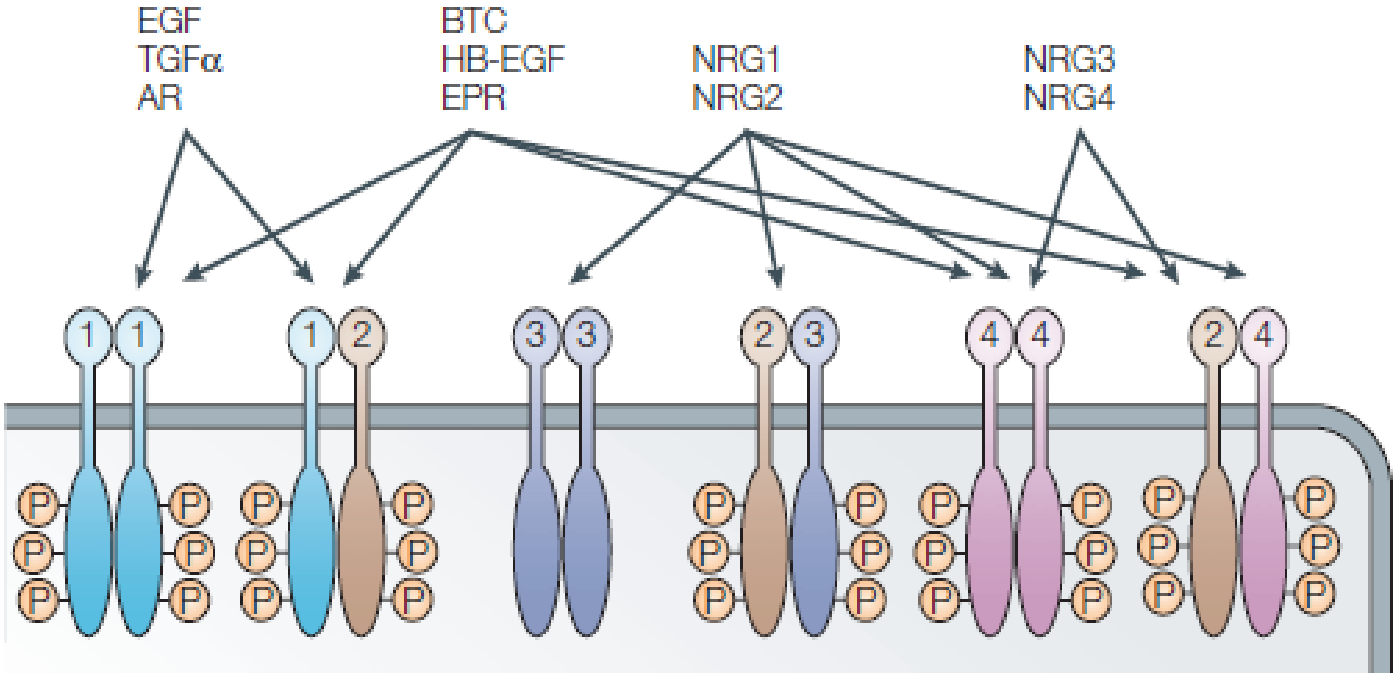


scFv-5R



ESMO

Ligand binding induces receptor dimerization

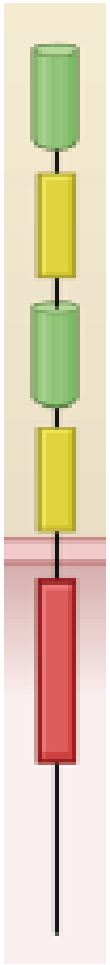


Hynes & Lane 2005 Nature Rev Cancer

ErbB2 is the preferred dimerization partner for the other ligand bound ErbBs.

ErbB2-containing heterodimers propogate strong & sustained signaling.

ErbB receptor activation in cancer



ErbBs

Receptor amplification/overexpression

ERBB2- amplification in breast, ovarian, gastric, endometrial, NSCLC, bladder, oropharyngeal, CRC….

EGFR- amplification in glioblastoma, SCCHN, esophageal, CRC…

ERBB3 – overexpression in many human tumors

Activating point mutations or deletions

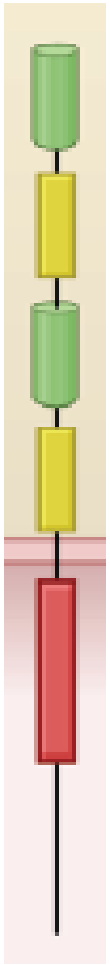
EGFR – glioblastoma, NSCLC, CRC….

ERBB2- breast, NSCLC, gastric, CRC…

ERBB3- gastric, CRC..

ERBB4 – CRC, melanoma …

ErbB receptor activation in cancer



ErbBs

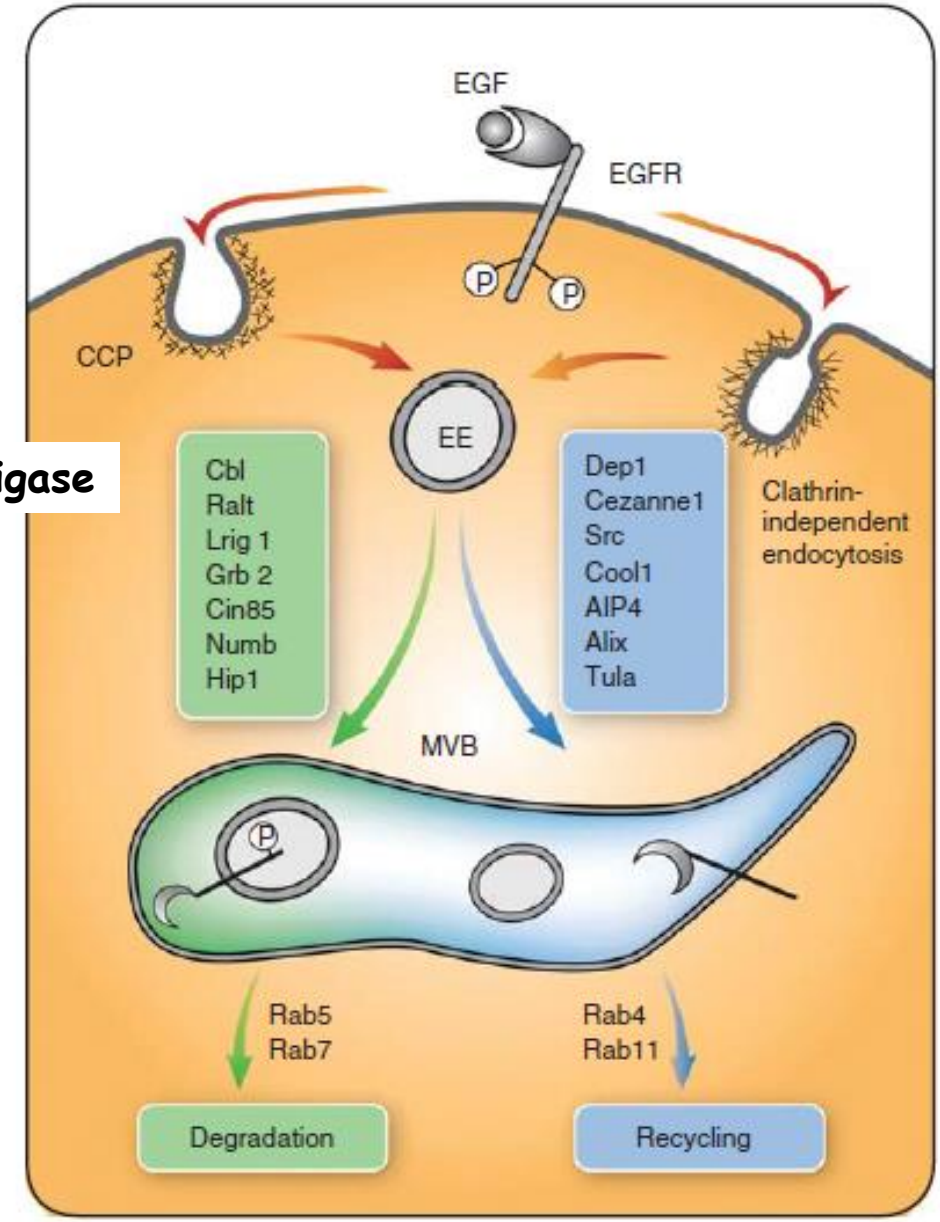
Mutant receptors have constitutive kinase activity leading to many alterations in tumor cells:

- altered patterns of receptor phosphorylation will cause changes in binding of signaling proteins & changes in pathway activity
- constitutive activation of downstream signaling
- evasion of negative regulators
- defects in receptor turnover/degradation
- etc…

Negative regulation of ErbB RTK signaling

Cbl- ubiquitin ligase

MIG6/RALT



Mellman & Yarden, 2013 CSHPB

ErbB2 & Breast Cancer

Breast cancer patients with the *ERBB2* amplicon have aggressive disease with poor clinical prognosis = metastasis.

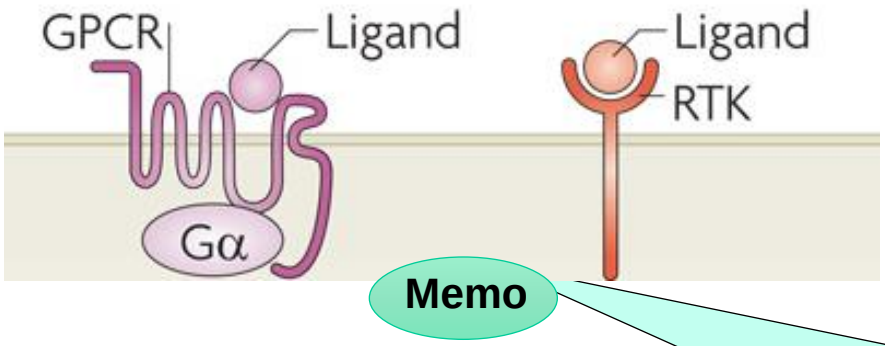
Goal – uncover ErbB2 interacting proteins with roles in migration & metastasis

Susanne Lienhard

Romina Marone

Ali Badache

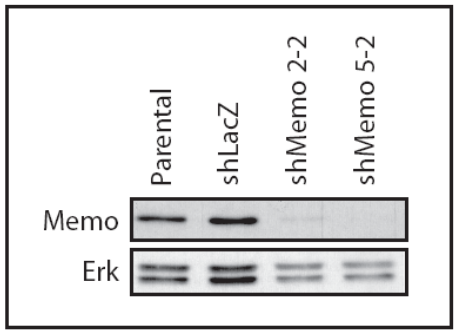
Memo - Summary

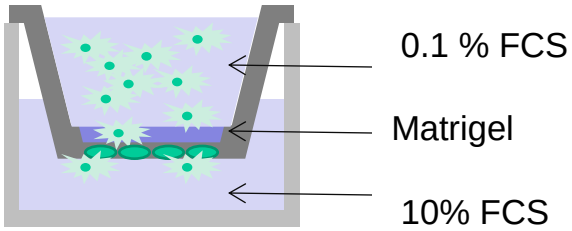


- **The 32kDa Memo (mediator of motility) was identified in a screen for proteins associated with ligand-activated ErbB2.**
- **Memo is required for migration in response to extracellular stimuli that activate RTKs & GPCRs (HRG, EGF, FGF, serum….).**
- **Memo function? Is Memo required for metastasis? Memo & breast cancer?**

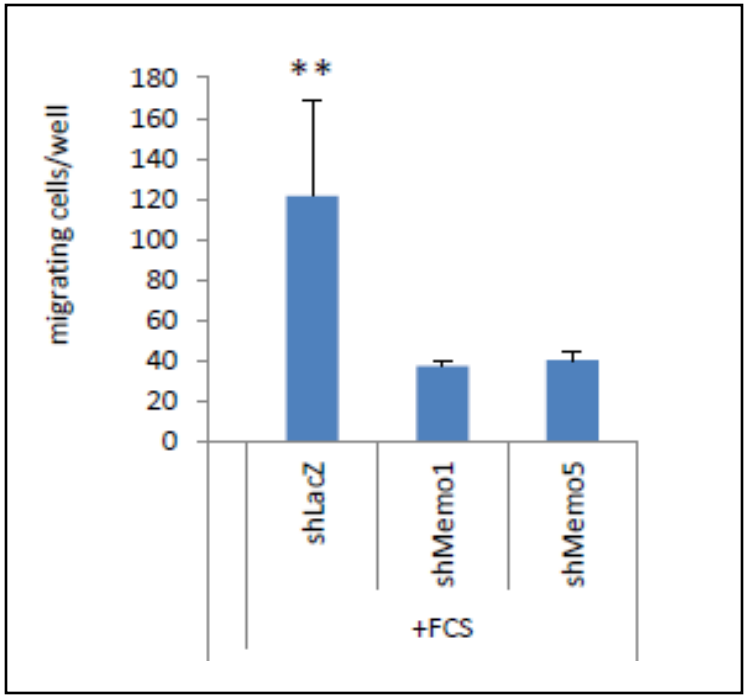
Marone et al 2004 NCB; Meira et al 2009 JCS; Haenzi et al 2013 Faseb J; Kondo et al 2014 PlosOne; MacDonald et al 2014 Science Signal

Memo KD decreases invasion & migration of MDA-MB-231 breast tumor cells





Memo KD MDA-MB-231 cells

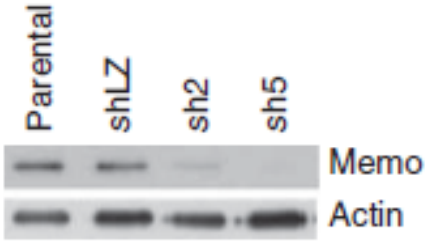


Gwen MacDonald

Is Memo required for metastasis?

MDA-MB231 human tumor cells:
aggressive metastatic breast cancer model

Stable Memo KD cell lines were generated & tested in vivo for their metastatic potential.



Gwen MacDonald

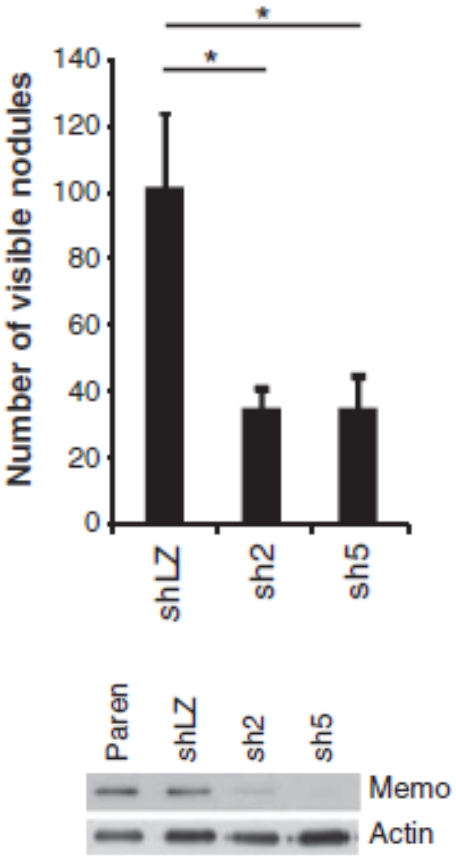
Tatiana Smirnova

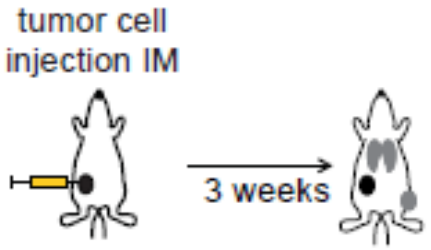
Anna Frei

MacDonald, Nalvarte et al 2014 Science Signal

Memo is required for efficient metastatic dissemination from primary tumors to the lungs

number of lung metastases

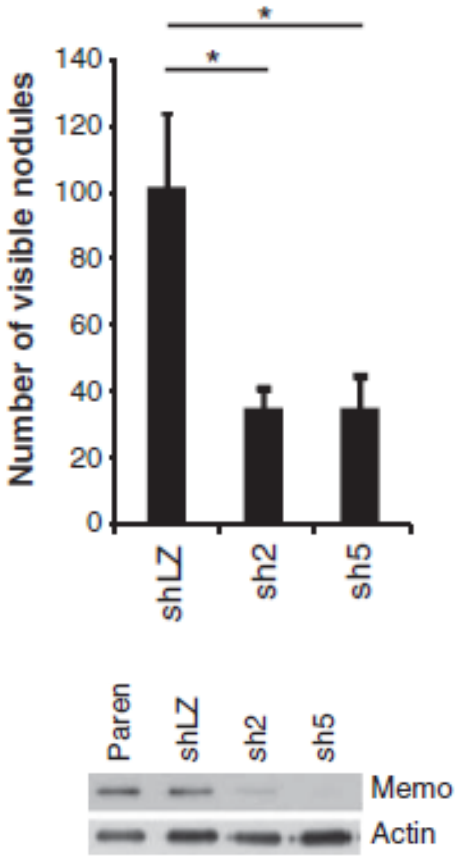




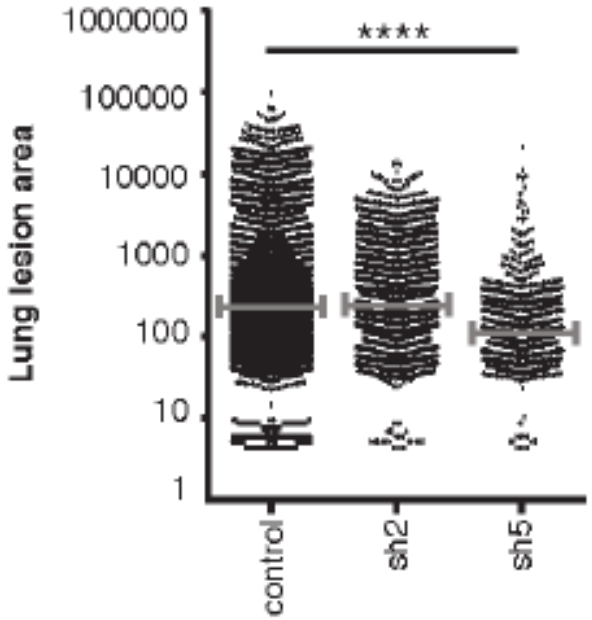
MacDonald, Nalvarte et al 2014 Science Signal

Memo KD lesions in the lungs are smaller; Memo loss effects proliferation in the metastatic site

number of lung metastases



lung lesion area



MacDonald, Nalvarte et al 2014 Science Signal

Memo is overexpressed in breast cancer

IHC analysis of >400 primary tumors

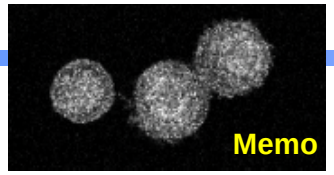
Memo levels are significantly increased in 43.7% primary breast tumors

Total Memo Expression

Tissue Type	Low (≤ 1.0)	High (> 1.0)	Total	p-value
Normal breast	29 (90.6%)	3 (9.4%)	32	0.0001
Breast cancer	229 (56.3%)	178 (43.7%)	407	

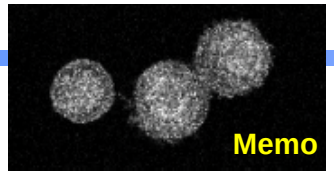
Is Memo a prognostic factor in breast cancer?

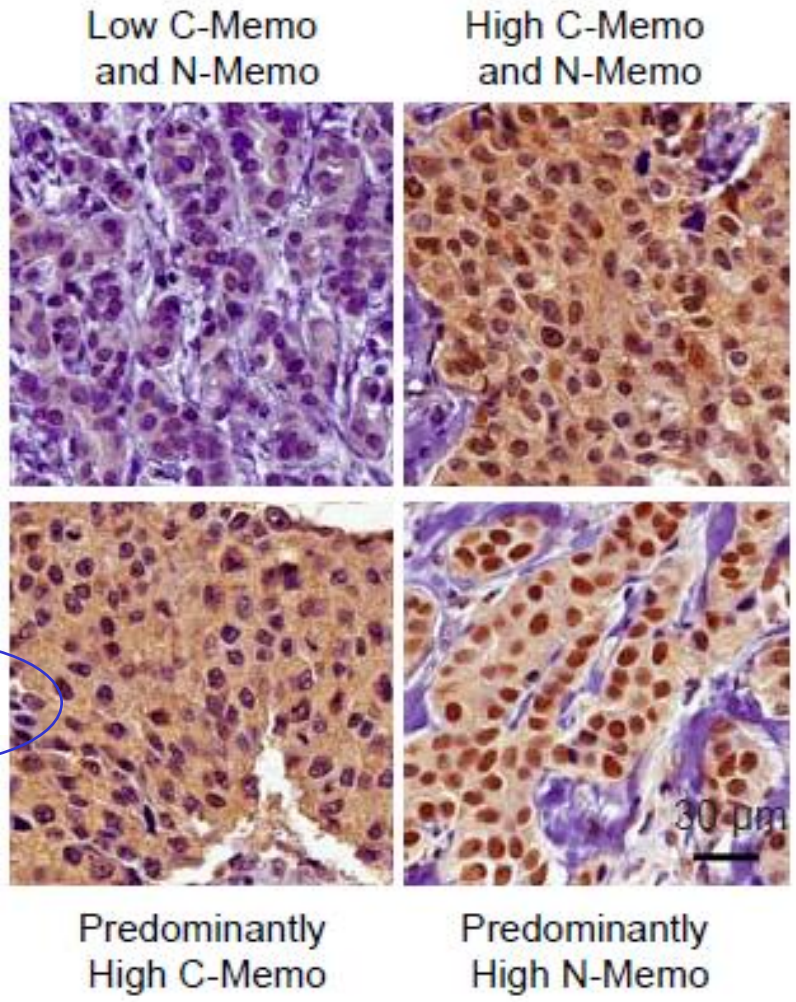
MacDonald, Nalvarte et al 2014 Science Signal



Memo is present in the cytoplasm & the nucleus of normal & cancer cells

Memo IHC- tumors scored for staining intensity and cellular localization





MacDonald, Nalvarte et al 2014 Science Signal

Manuela Vecchi
Paolo Di Fiore
IFOM, Milan

High cytoplasmic-Memo correlates with poor prognostic factors & with aggressive breast cancer sub-types

Variable	C-Memo (*) High vs Low OR	p
Grade		
2 vs 1	1.8	0.07
3 vs 1	2.93	0.007
ER/PgR - vs +	3.91	0.004
ErbB2 + vs -	3.36	0.028
Ki-67 ≥14% vs <14%	2.17	0.005
p53 ≥16% vs <16%	3.03	0.001
Subtype		
Lum B Ki67 High vs Lum A	1.69	0.07
Lum B ErbB2+ vs Lum A	5.51	0.012
ErbB2+ vs Lum A	4.55	0.139
TN vs Lum A	6.32	0.001

OR – overall survival

High nuclear grade
High ErbB2
ER/PgR negative
High Ki67 & p53
High Triple Negative

High nuclear Memo has an inverse relationship with these factors, i.e. it correlates with better prognosis.

MacDonald, Nalvarte et al 2014 Science Signal

High cytoplasmic-Memo predicts early relapse and death (5 ≤ years)

	Distant metastasis				Overall survival			
	C-Memo High vs Low		N-Memo High vs Low		C-Memo High vs Low		N-Memo High vs Low	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
≤ 5 years	3.89 (1.16;13.07)	0.028	0.44 (0.13;1.53)	0.2	8.36 (1.31;53.42)	0.025	0.38 (0.07;2.1)	0.27
> 5 years	0.44 (0.11;1.8)	0.25	0.57 (0.16;2.01)	0.38	1.05 (0.45;2.43)	0.92	0.54 (0.23;1.28)	0.16

Multivariable Cox proportional hazards model

TMA analysis on primary breast tumors:

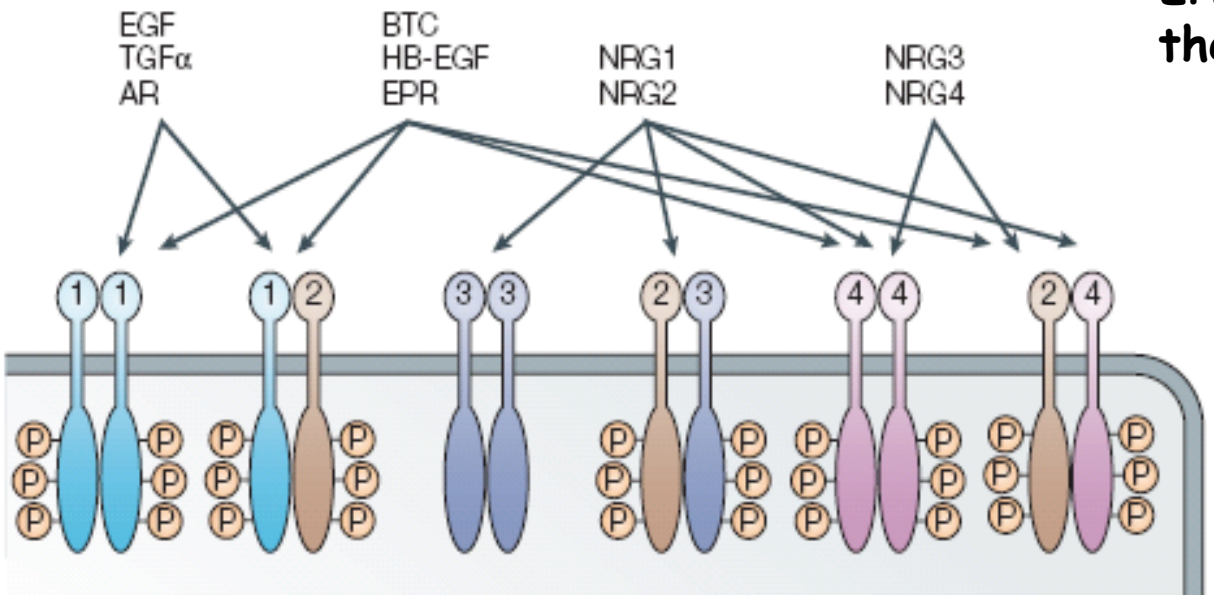
Memo might have distinct roles in the cytoplasm & the nucleus.

Cytoplasmic function regulates pathways that promote tumor metastasis, contributing to poor prognosis.

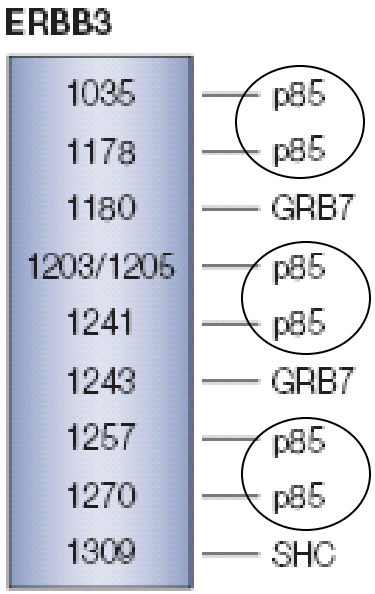
MacDonald, Nalvarte et al 2014 Science Signal

ErbB2/ErbB3 Heterodimer in Cancer

- ErbB3 has "impaired" kinase activity.
- ErbB2/ErbB3 heterodimer has potent transforming activity.



ErbB3 strongly activates the PI3K pathway



PNAS | July 22, 2003 | vol. 100 | no. 15 | 8933–8938

The ErbB2/ErbB3 heterodimer functions as an oncogenic unit: ErbB2 requires ErbB3 to drive breast tumor cell proliferation

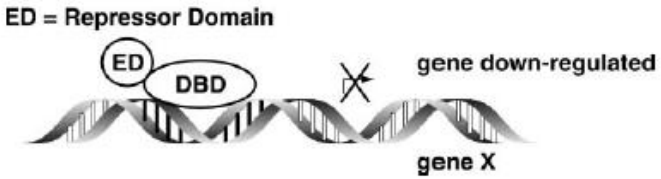
Thomas Holbro*, Roger R. Beerli[†‡], Francisca Maurer*, Magdalena Koziczak*, Carlos F. Barbas III[†], and Nancy E. Hynes*[§]

*Friedrich Miescher Institute, P.O. Box 2543, 4002 Basel, Switzerland; and [†]The Skaggs Institute for Chemical Biology and Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037

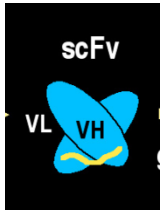
Thomas Holbro

E3- Designer Transcription factor to block ErbB3 transcription

Carlos Barbas III (1965-2014)



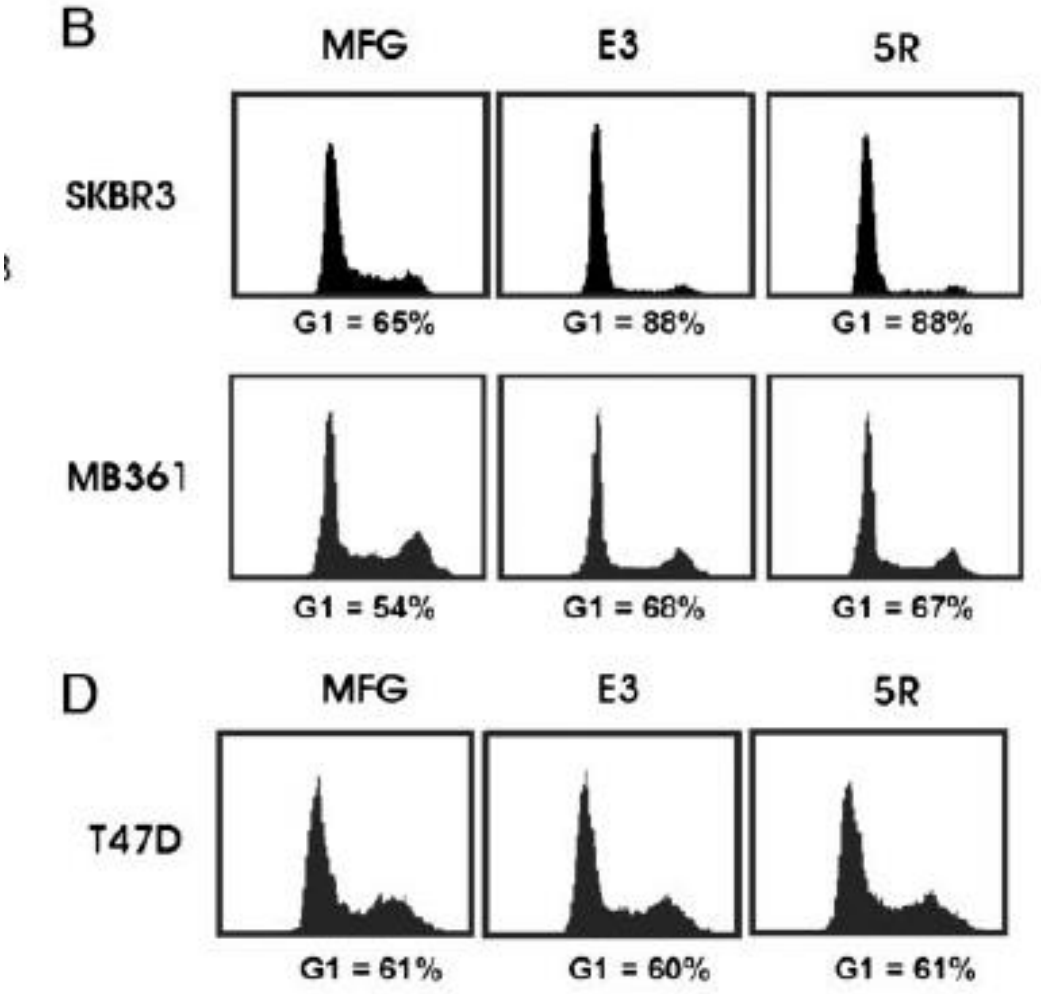
5R single chain Ab for ErbB2 ER retention



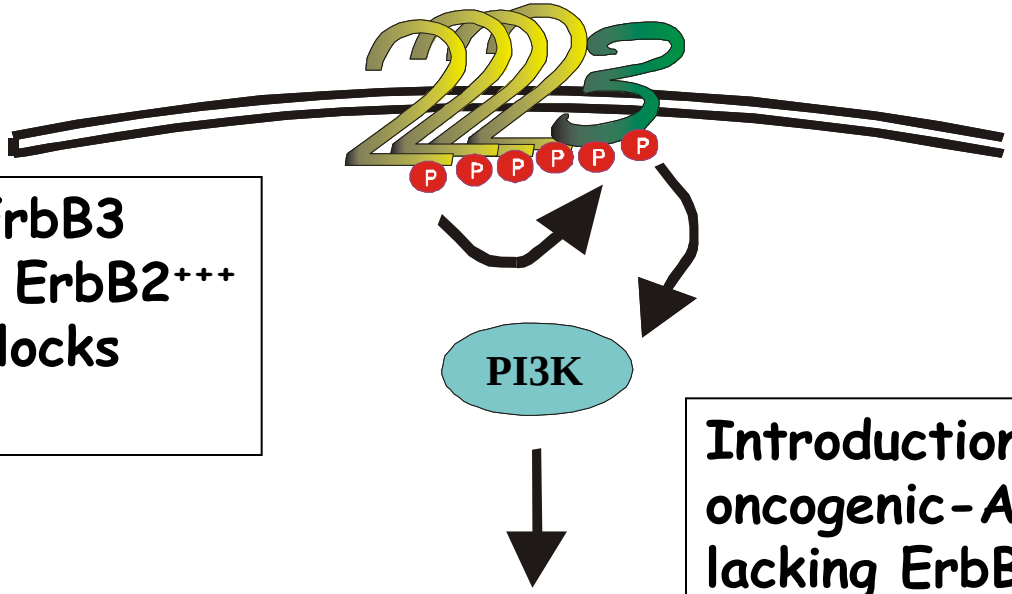
Loss of ErbB3 or ErbB2 blocks proliferation of ErbB2+++ tumor cells

ErbB2+++

ErbB2



ErbB2 requires ErbB3 to drive proliferation



Ablation of ErbB3 expression in ErbB2^{+++} tumor cells blocks proliferation.

Introduction of oncogenic-Akt into cells lacking ErbB3 restores proliferation.

ErbB3 functions to link ErbB2 to the PI3K/Akt pathway

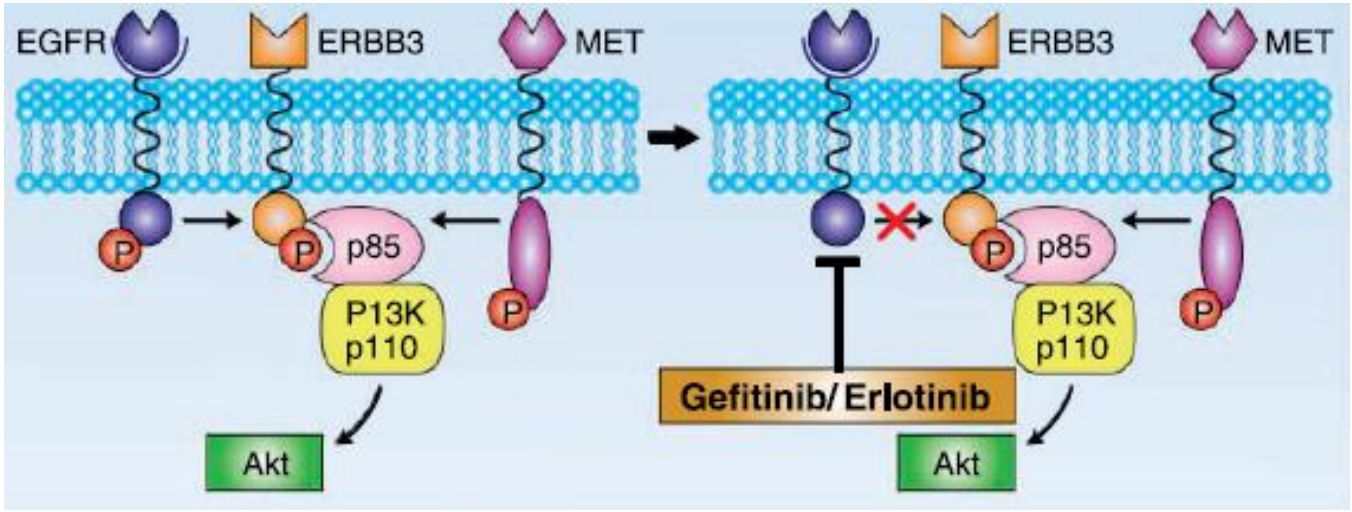
Holbro et al 2003 PNAS

Other RTKs use ErbB3 to escape targeted therapies

Mechanisms of Acquired Resistance to Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors in Non–Small Cell Lung Cancer

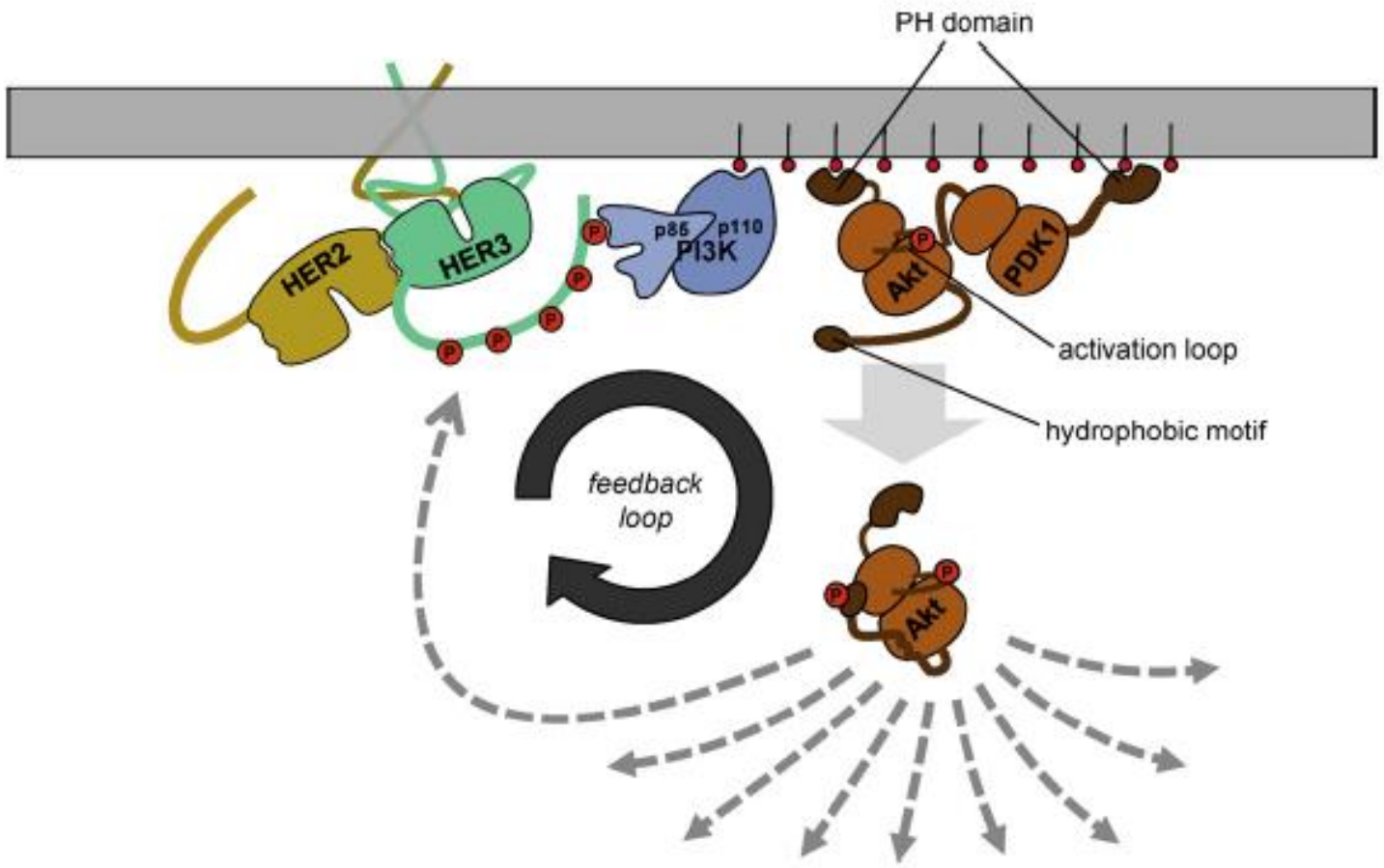
Jeffrey A. Engelman[1] and Pasi A. Jänne[2,3]

Clin Cancer Res 2008;14(10) May 15, 2008



MET receptor in NSCLC

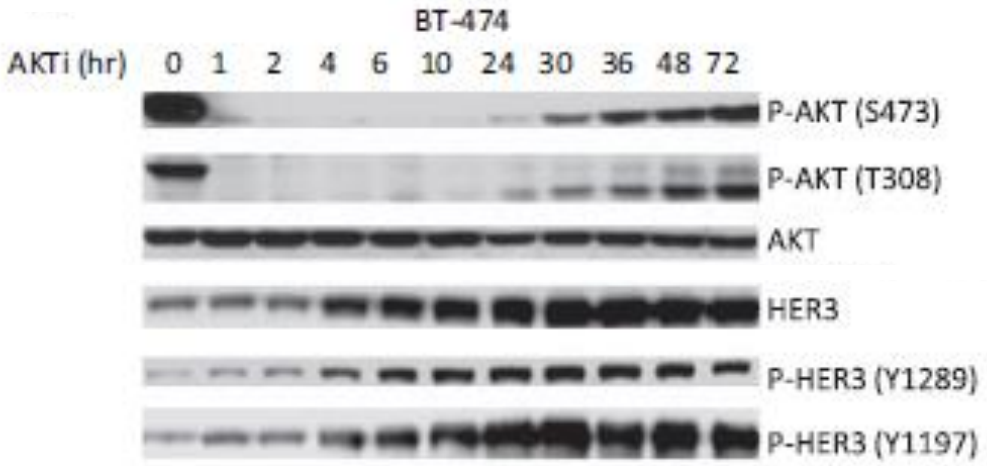
Moasser & colleagues showed in 2007 that high ErbB3 activity promoted escape from ErbB2 targeted therapeutics (Sergina et al 2007)



D. Amin et al 2010

Blockade of PI3K/AKT promotes ErbB3 expression & phosphorylation

AKT inhibitor



Chandarlapaty et al 2011

AKT dependent feed-back mechanisms in cancer cells

Constitutive feedback inhibition of signaling pathways is an important feature of tumors…. allowing them to escape stress-related processes that would cause cell death.

AKTi relieves negative feedback inhibition…

Chandarlapaty et al 2011

AKT dependent feed-back mechanisms in cancer cells

Constitutive feedback inhibition of signaling pathways is an important feature of tumors…. allowing them to escape stress-related processes that would cause cell death.

AKTi relieves negative feedback inhibition…

Phospho-RTK-Fold induced

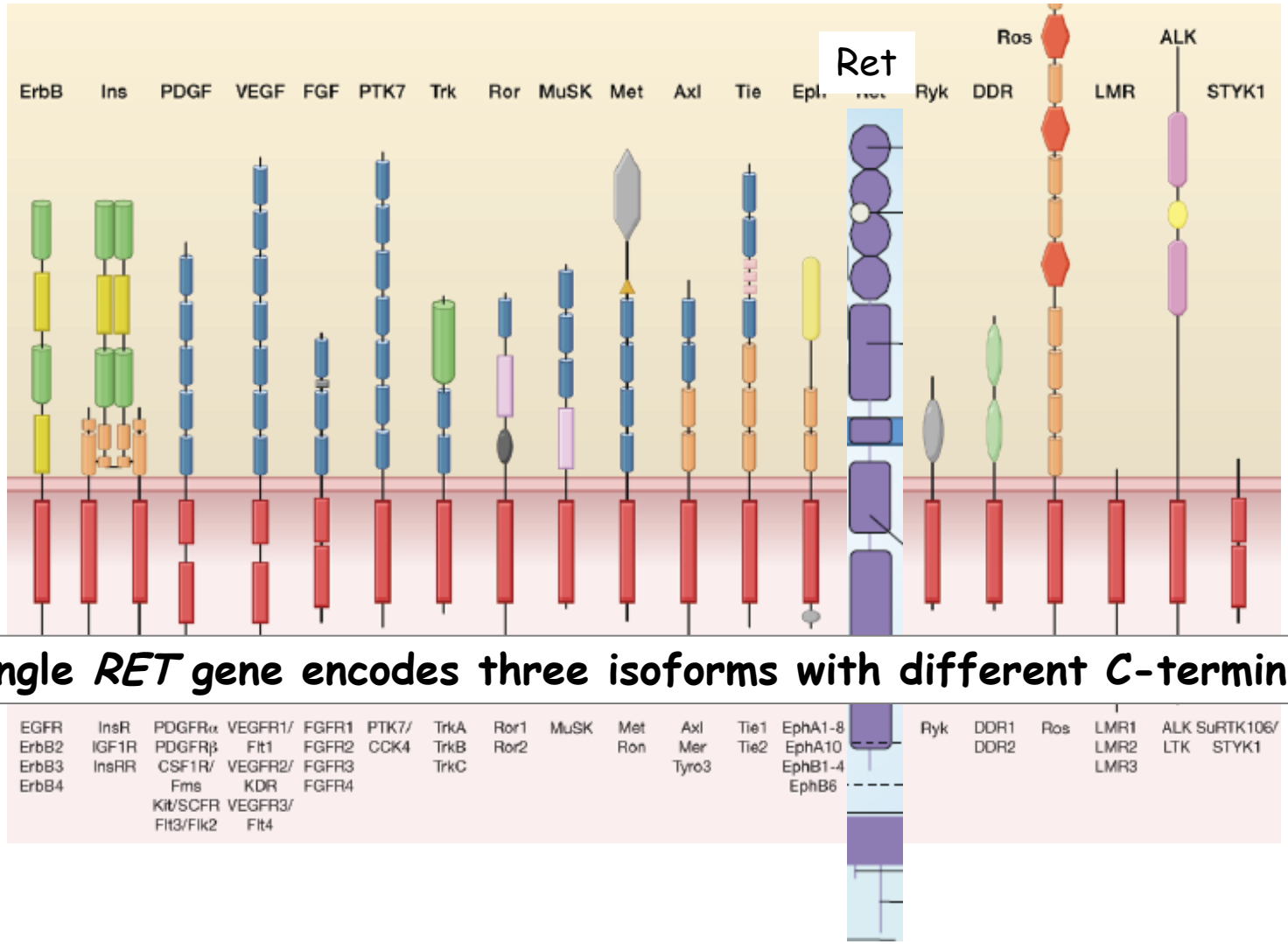
P-RTK	mean induced	#cell lines
Insulin R	5.0	10/10
HER3	5.5	8/10
IGF-1R	4.8	8/10
EphA7	8.4	7/10
c-RET	5.5	5/10
HER4	3.3	5/10
EphA1	3.1	5/10
ROR1	3.1	4/10
C-MET	3.0	4/10

…leading to activation of multiple RTKs by different mechanisms.

ErbB3, INSR & IGF-1R are transcriptionally upregulated by FOXO.

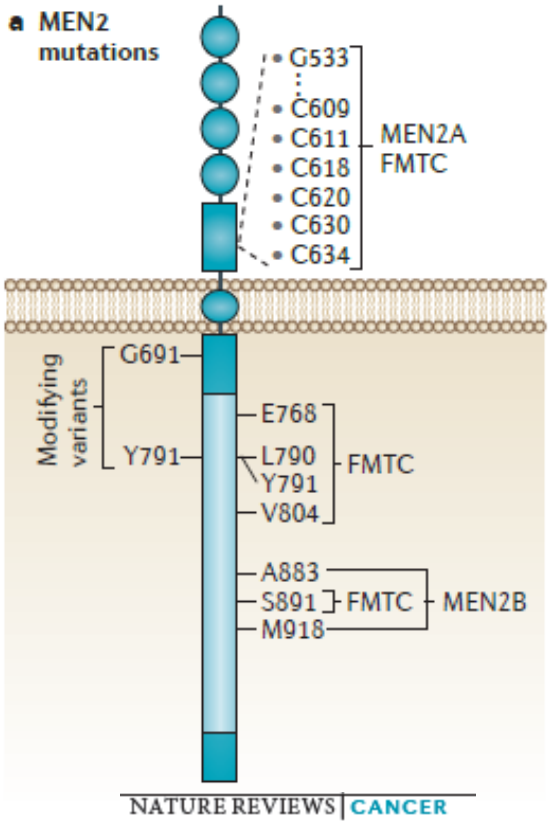
Chandarlapaty et al 2011

RET receptor in breast cancer



Ret Receptor – Oncogenic mutations

Heritable Ret mutations in MEN2
(Multiple endocrine neoplasia 2)

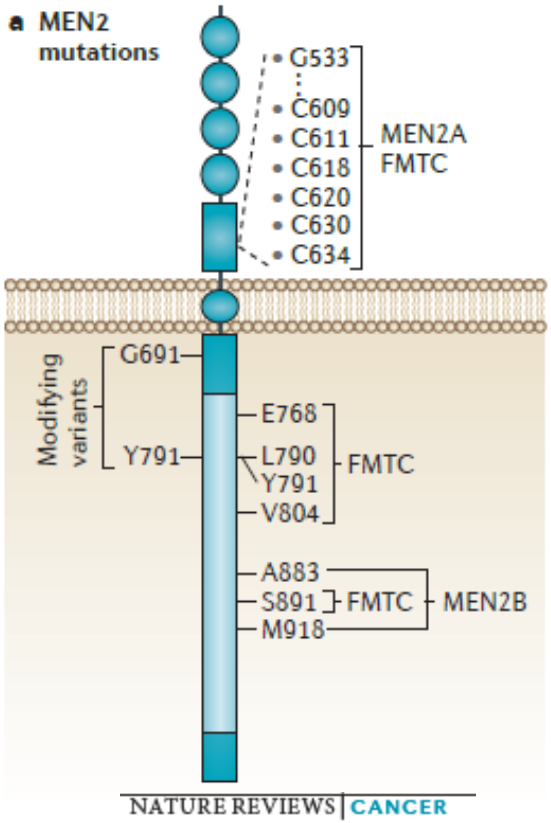


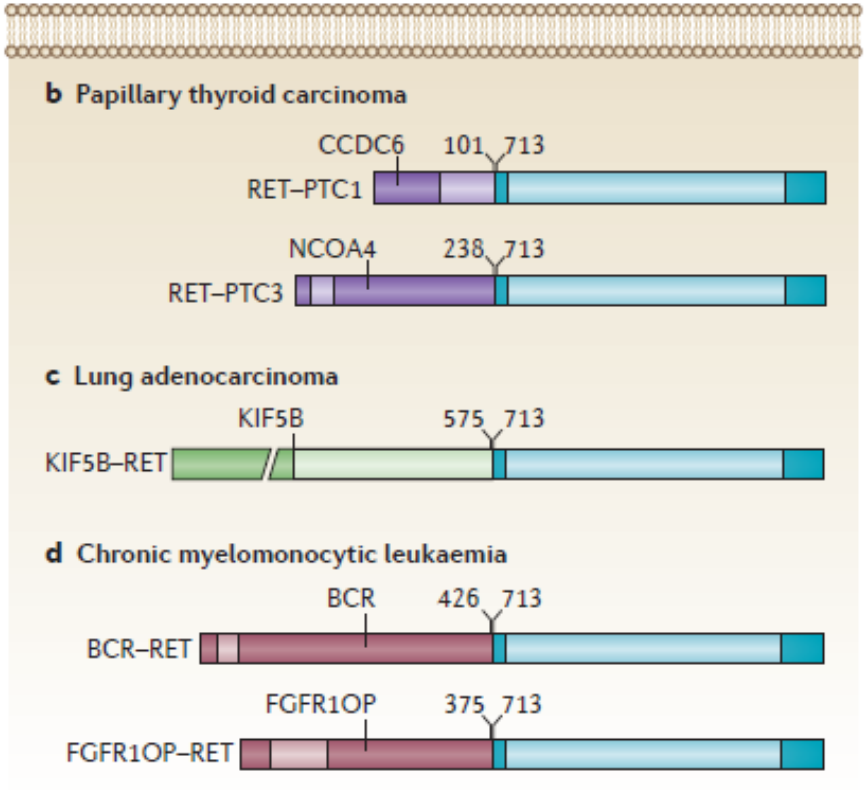
L Mulligan 2014

Ret Receptor – Oncogenic mutations

Heritable Ret mutations in MEN2 (Multiple endocrine neoplasia 2)

Somatic Ret mutations –fusion proteins





L Mulligan 2014

Ret receptor alterations in breast cancer

Breast Cancer

RET copy number changes - rare

RET rearrangements - a few reported

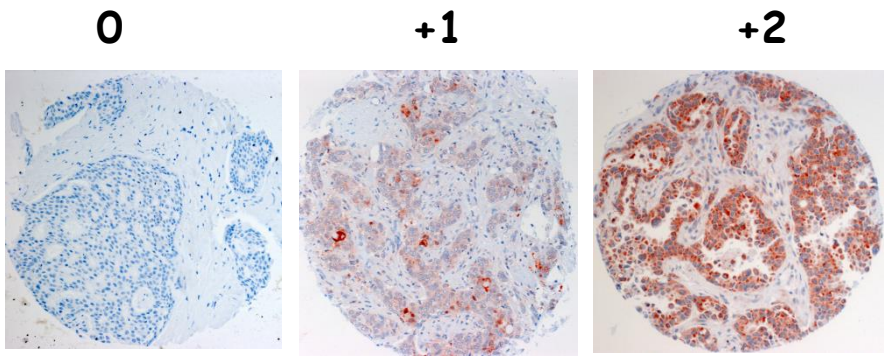
Ret activating mutations - none

Elevated Ret RNA & protein levels – common

Essighir et al Cancer Res 2007
Boulay et al Cancer Res 2008
Plaza-Menacho et al Oncogene 2010
Kothari et al Cancer Dis 2013
Gattelli et al EMBO Mol Med 2013
Stransky et al Nature Comm 2014

IHC for Ret in a breast tumor TMA

Elevated Ret levels detected in >60% of tumors (+1 and +2)



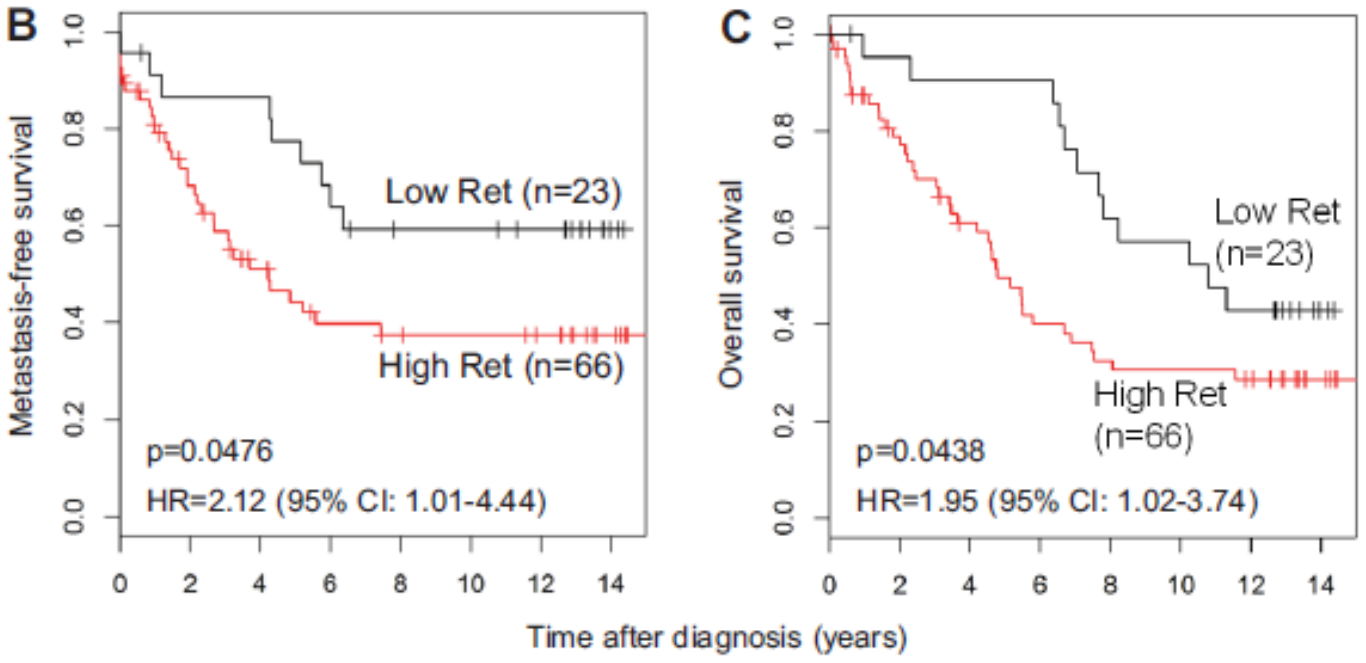
Breast Tumor Tissue Array (TMA) n=108

ER+ luminal tumors, ErbB2+++ and triple negative tumors show elevated Ret levels.

Gattelli et al 2013

Elevated Ret levels correlate with poor prognosis

Kaplan-Meier analyses of metastasis-free survival & overall survival



No. at risk								
Low Ret	23	19	19	15	11	11	9	2
High Ret	66	37	24	17	15	14	12	5

No. at risk								
Low Ret	23	20	19	19	13	12	9	2
High Ret	66	45	32	21	17	16	14	5

Gattelli et al 2013

Conclusions & questions

WT Ret has oncogenic activity when constitutively expressed in the mammary gland.

Ret is a potential novel target in breast cancer.

Does Ret activity influence response to endocrine agents? (Morandi et al 2013 Can Res)

What are the signals that upregulate Ret levels? epigenetic, promoter mutations…..

Overview of ErbB receptors

Mutations in human cancers

Activation mechanisms
Role of ErbB2
ErbB2/ErbB3 heterodimers

Novel downstream regulator of metastasis

RET receptor and breast cancer