Therapeutic Strategies for Human Papillomavirus-Associated Cancers

Karl Münger

The Channing Laboratory,
Infectious Diseases Division
Brigham and Women's Hospital,
Boston, MA 02115

kmunger@rics.bwh.harvard.edu

Why consider therapeutic approaches for HPV-associated lesions and cancers?

500,000 new cases/288,000 cervical cancer deaths per year (worldwide)

(2nd most common cause of cancer death in women)

Prophylactic vaccines will only protect from new infections by vaccine HPVs

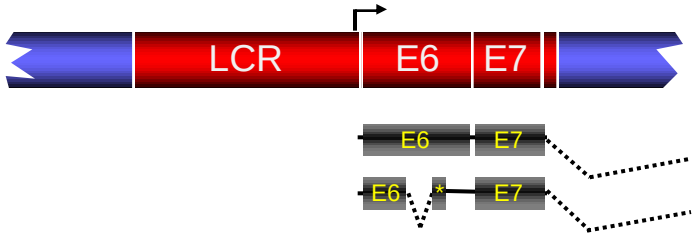
Vaccine implementation has been challenging.

(US 2007: 25% of girls ages 13-17, 10% of all females ages 18-26
1.1% of Hispanic women)

Cervical cancer develops decades after the initial HPV infection

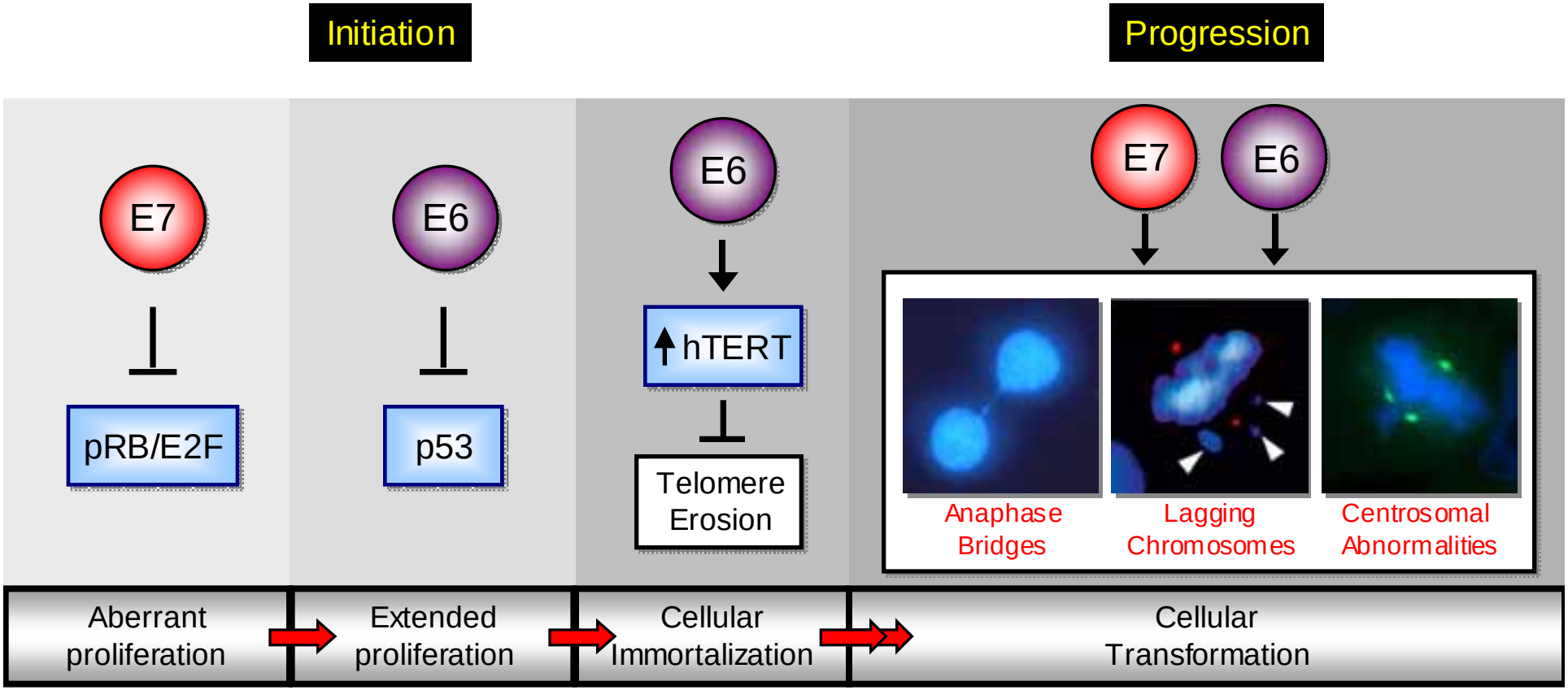
It will take decades before such vaccines will result in a measurable decline of HPV-associated cancers

HPV genome integration is a hallmark of malignant progression



HPV-associated cancers only express two viral proteins, E6 and E7

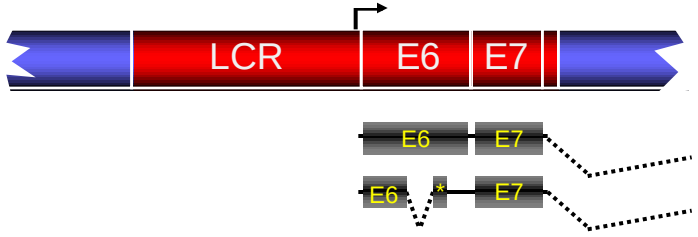
Mechanisms of HPV-associated carcinogenesis



HPV oncoproteins target cellular signaling pathways that are frequently mutated in human solid tumors:

pRB pathway >80%
p53 >60%
Telomere maintenance ~100%

Evidence that HPV E6/E7 expression is necessary and sufficient for cervical carcinogenesis



HPV16 E6/E7 expression in primary epithelial cells causes histopathological abnormalities reminiscent of CIN3

HPV16 E6/E7 expression causes cervical cancer in transgenic mice

Extinguishing HPV E6/E7 expression in cervical cancer cell lines causes cellular senescence/G1 arrest/apoptosis

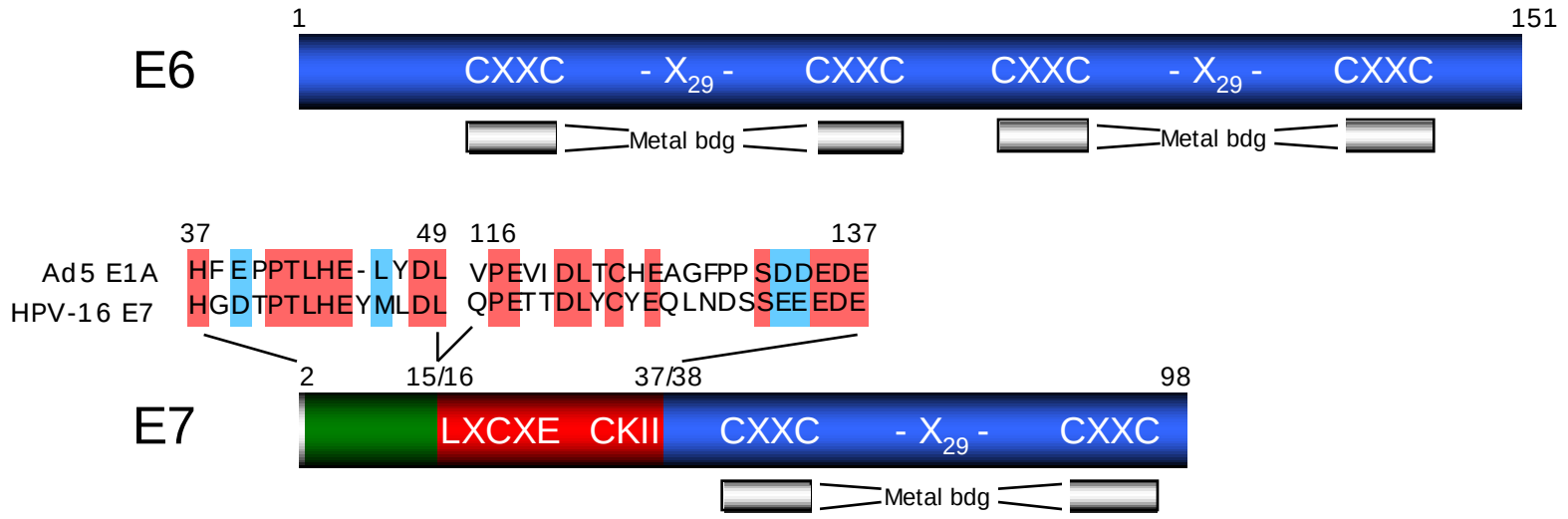
HPV-associated cancers are the only human solid tumors where the carcinogenic agent is known at a molecular level

Therapeutic opportunities

HPV-associated cancers are driven by expression of the E6 and E7 oncoproteins

HPV E6 and E7 oncoproteins should be excellent drug targets

HPV E6/E7 Oncoproteins



No cellular homologues

No intrinsic enzymatic activities

No specific DNA binding activities

Associate with and functionally modify host cellular protein complexes

Therapeutic opportunities

HPV E6 and E7 function through protein/protein interactions
(E6/p53; E7/pRB)

Protein/protein interactions are difficult to target by small molecule approaches

Identification of therapeutic targets

Are HPV oncoproteins associated with cellular enzymatic activities that are necessary for their activities?

Does expression of HPV oncoproteins induce perturbations of cellular signal transduction networks that may be harnessed for therapy?

Identification of therapeutic targets

Are HPV oncoproteins associated with cellular enzymatic activities that are necessary for their activities?

Proteomic analysis of HPV16 E7 associated host cellular protein complexes

Construction of stable cell lines expressing double tagged E7

↓

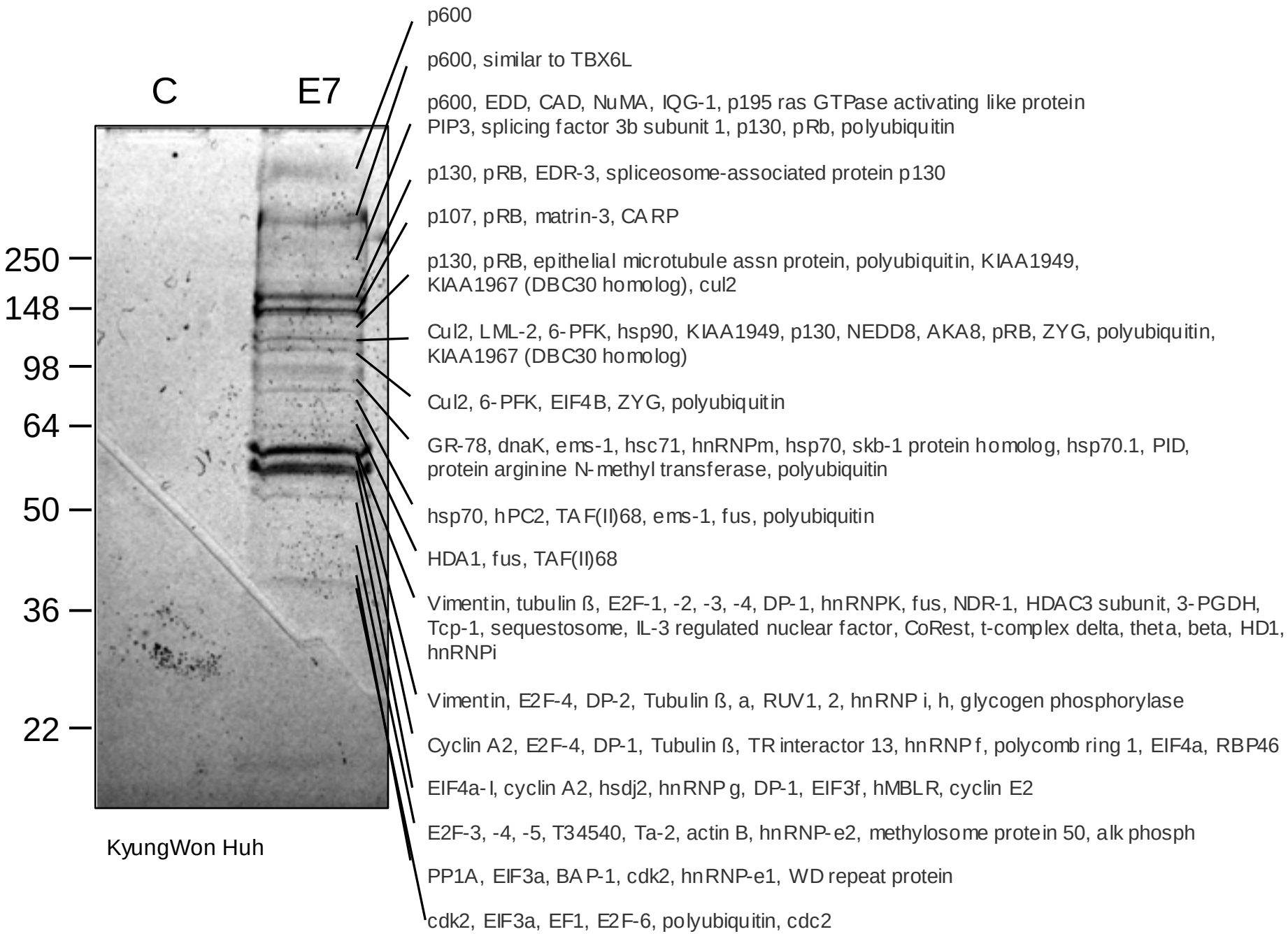
Sequential immunoaffinity purification with anti-FLAG and anti-HA resin

↓

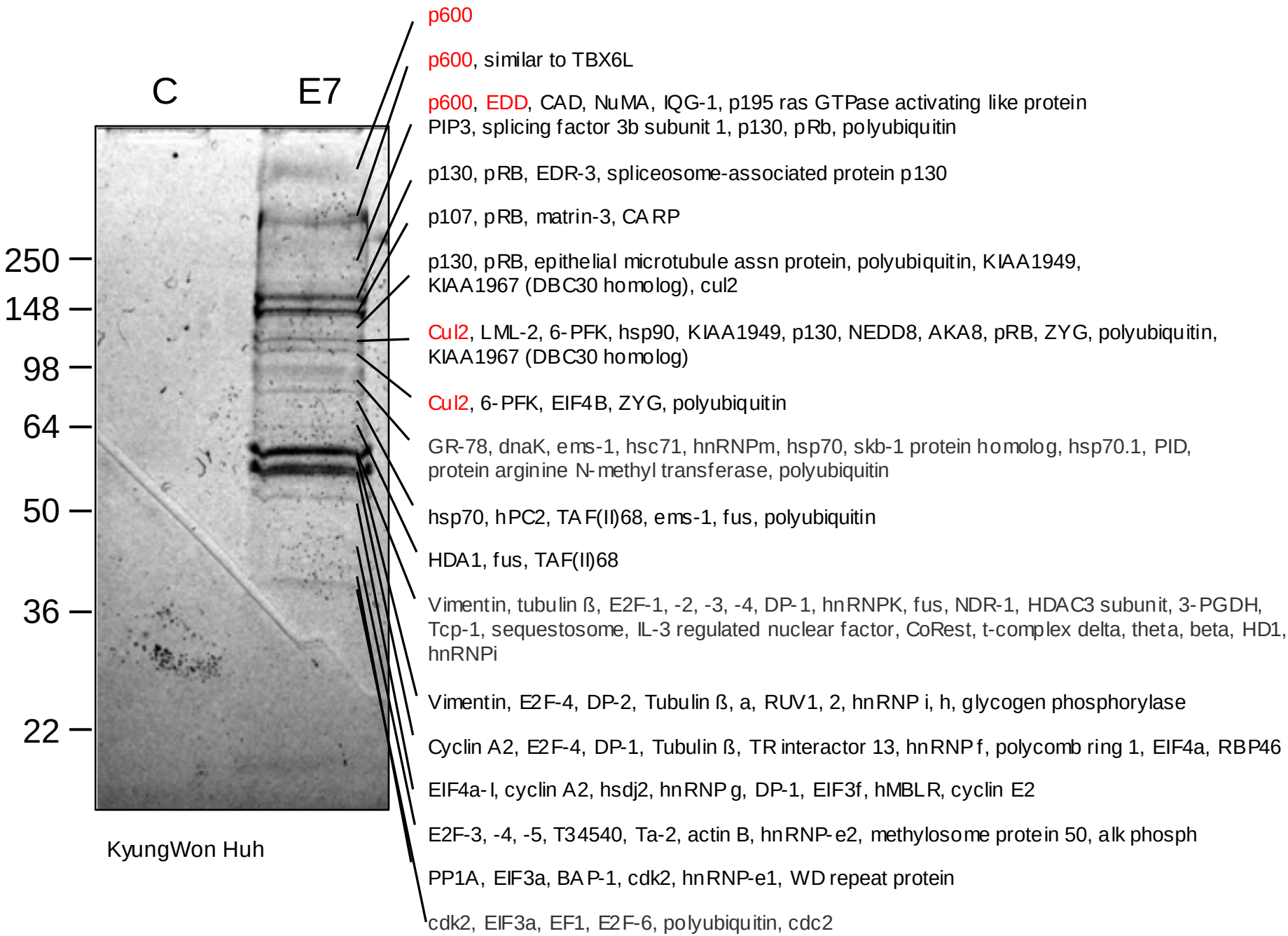
SDS-PAGE and stain

↓

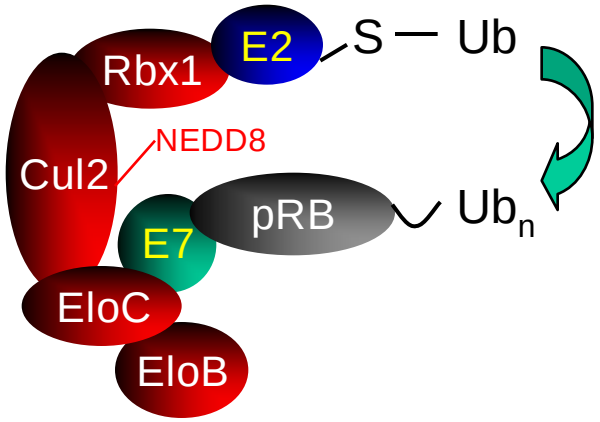
Identification of protein components by mass spectrometry



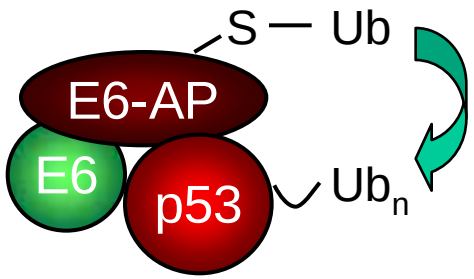
KyungWon Huh


C
E7
250
148
98
64
50
36
22
p600
p600, similar to TBX6L
p600, EDD, CAD, NuMA, IQG-1, p195 ras GTPase activating like protein PIP3, splicing factor 3b subunit 1, p130, pRb, polyubiquitin
p130, pRB, EDR-3, spliceosome-associated protein p130
p107, pRB, matrin-3, CARP
p130, pRB, epithelial microtubule assn protein, polyubiquitin, KIAA1949, KIAA1967 (DBC30 homolog), cul2
Cul2, LML-2, 6-PFK, hsp90, KIAA1949, p130, NEDD8, AKA8, pRB, ZYG, polyubiquitin, KIAA1967 (DBC30 homolog)
Cul2, 6-PFK, EIF4B, ZYG, polyubiquitin
GR-78, dnaK, ems-1, hsc71, hnRNPm, hsp70, skb-1 protein homolog, hsp70.1, PID, protein arginine N-methyl transferase, polyubiquitin
hsp70, hPC2, TAF(II)68, ems-1, fus, polyubiquitin
HDA1, fus, TAF(II)68
Vimentin, tubulin ß, E2F-1, -2, -3, -4, DP-1, hnRNPK, fus, NDR-1, HDAC3 subunit, 3-PGDH, Tcp-1, sequestosome, IL-3 regulated nuclear factor, CoRest, t-complex delta, theta, beta, HD1, hnRNPi
Vimentin, E2F-4, DP-2, Tubulin ß, a, RUV1, 2, hnRNP i, h, glycogen phosphorylase
Cyclin A2, E2F-4, DP-1, Tubulin ß, TR interactor 13, hnRNP f, polycomb ring 1, EIF4a, RBP46
EIF4a-I, cyclin A2, hsdj2, hnRNP g, DP-1, EIF3f, hMBLR, cyclin E2
E2F-3, -4, -5, T34540, Ta-2, actin B, hnRNP-e2, methylosome protein 50, alk phosph
PP1A, EIF3a, BAP-1, cdk2, hnRNP-e1, WD repeat protein
cdk2, EIF3a, EF1, E2F-6, polyubiquitin, cdc2
KyungWon Huh

HPV E6 and E7 oncoproteins associate with and reprogram cellular enzymes



HPV16 E6 retargets the cellular cullin 2 ubiquitin ligase complex to the retinoblastoma tumor suppressor protein, pRB



HPV16 E6 retargets the cellular E6AP ubiquitin ligase to the p53 tumor suppressor protein

HPV E6 and E7 oncoproteins reprogram cellular ubiquitin ligases to target associated cellular tumor suppressors for degradation

Are HPV-positive cancer lines sensitive to the proteasome inhibitor, Bortezomib?

EC50s:

CaSki (HPV16): ~10-15 nM
SiHa (HPV16): ~30-35 nM
HeLa (HPV18): ~25 nM

Will test on HPV16 oncogene expressing primary human epithelial cells

Karin Hellner
collaboration with Jochen Lorch/Marshall Posner

Identification of therapeutic targets

Does expression of HPV oncoproteins induce perturbations of cellular signal transduction networks that may be harnessed for therapy?

"Under the Streetlight" Approach

"You study what you can see"

"Unbiased" approach

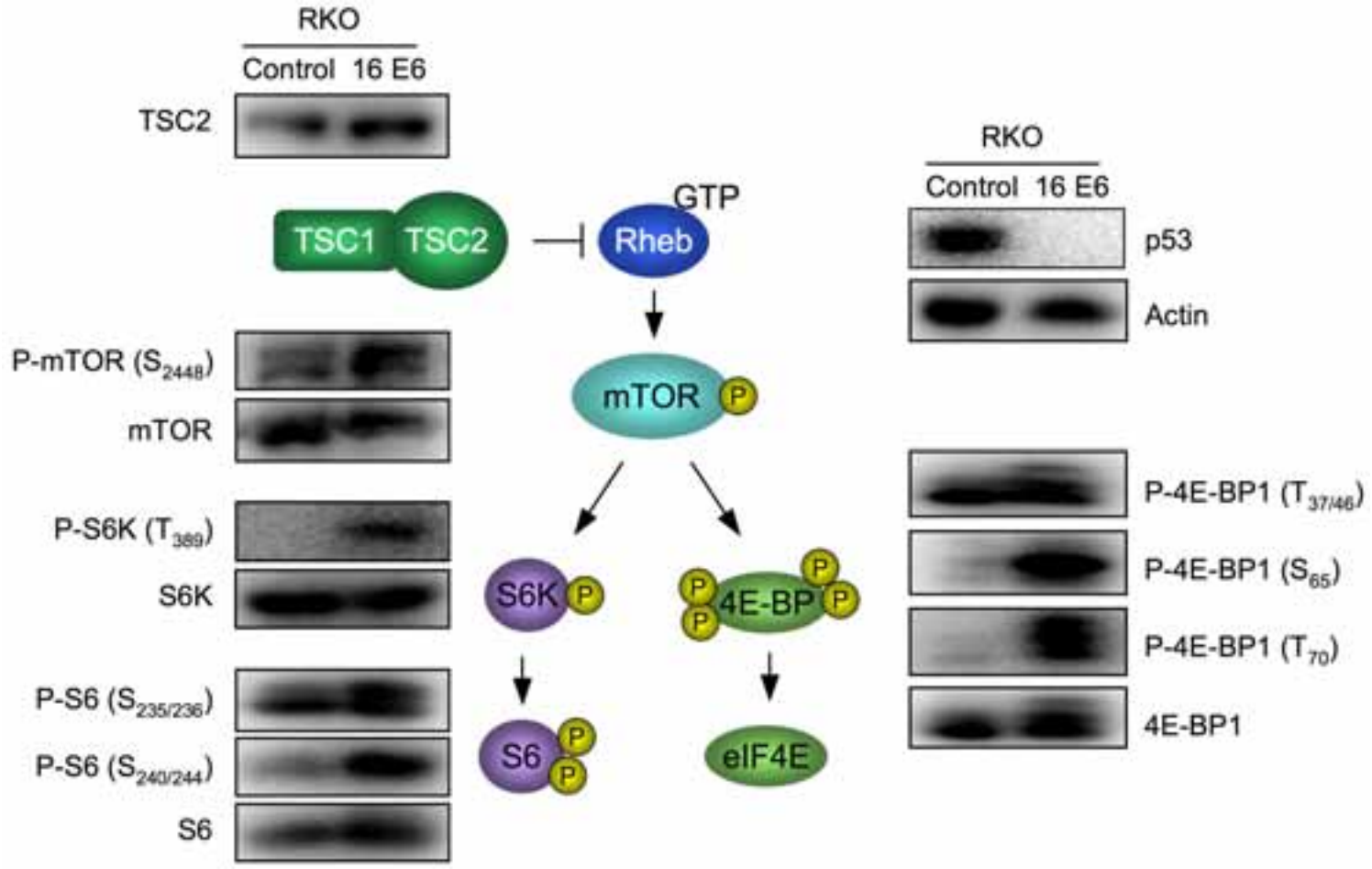
"You see what you can study"

Identification of therapeutic targets

"Under the Streetlight" Approach

"You study what you can see"

HPV E6 expression activates mTOR signaling



Jennifer Spangle based on
Lu et al, JBC 279: 35664-70, 2004

Are HPV-positive cancer lines susceptible to mTOR inhibitors?

EC50s for RAD001:

CaSki (HPV16): ~15 µM
SiHa (HPV16): ~25 µM
HeLa (HPV18): ~27 µM

Will test on HPV16 oncogene expressing primary human epithelial cells

Will test combination with 2-deoxyglucose

Karin Hellner
collaboration with Jochen Lorch/Marshall Posner

Identification of therapeutic targets

"Unbiased" approach

"You see what you can study"

Genetic Screens

Systems Biology

Identification of therapeutic targets

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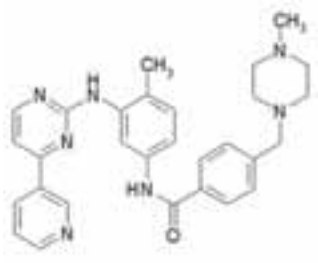
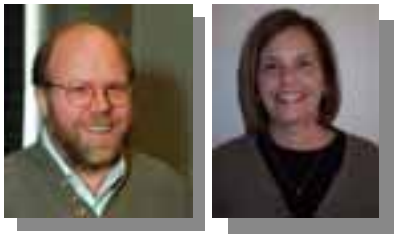
Genetic Screens

Essential kinases for cervical cancers

Precedence: Gleevec® inhibits the bcr/abl kinase that is critical for the growth of certain leukemias (CML) that have the Philadelphia translocation



Can we identify a kinase target for a “cervical cancer-specific Gleevec”?



Ed Harlow

Dorre Grueneberg

Karl Munger

Amy Baldwin

Karin Hellner

Miranda Grace

Haoxuan Tong

Essential kinases for cervical cancers: Experimental Strategy

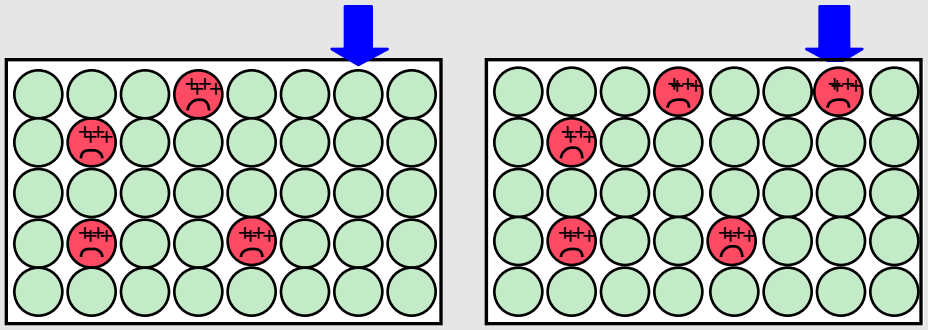
Normal Keratinocytes

Cervical Carcinoma

"100 Hits" Kinase shRNA library

Targets a subset of 80 kinases that potently kill a variety of cancer cell lines

Identify essential kinases for each cell population



Normal Keratinocytes

Cervical Carcinoma
ANPb
CDK7
EPHB1
HER3
HIPK2
IRR
JNK3
KHS1
MELK
MYO3B
PAK3
PAK6
PCTAIRE1
PDHK2
PITSLRE
PLK1
ROS
RSK2
SGK2
TSSK

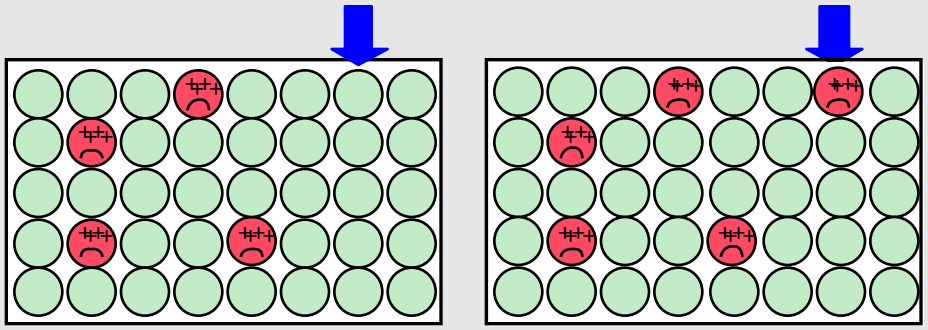
Essential kinases for cervical cancers: Experimental Strategy

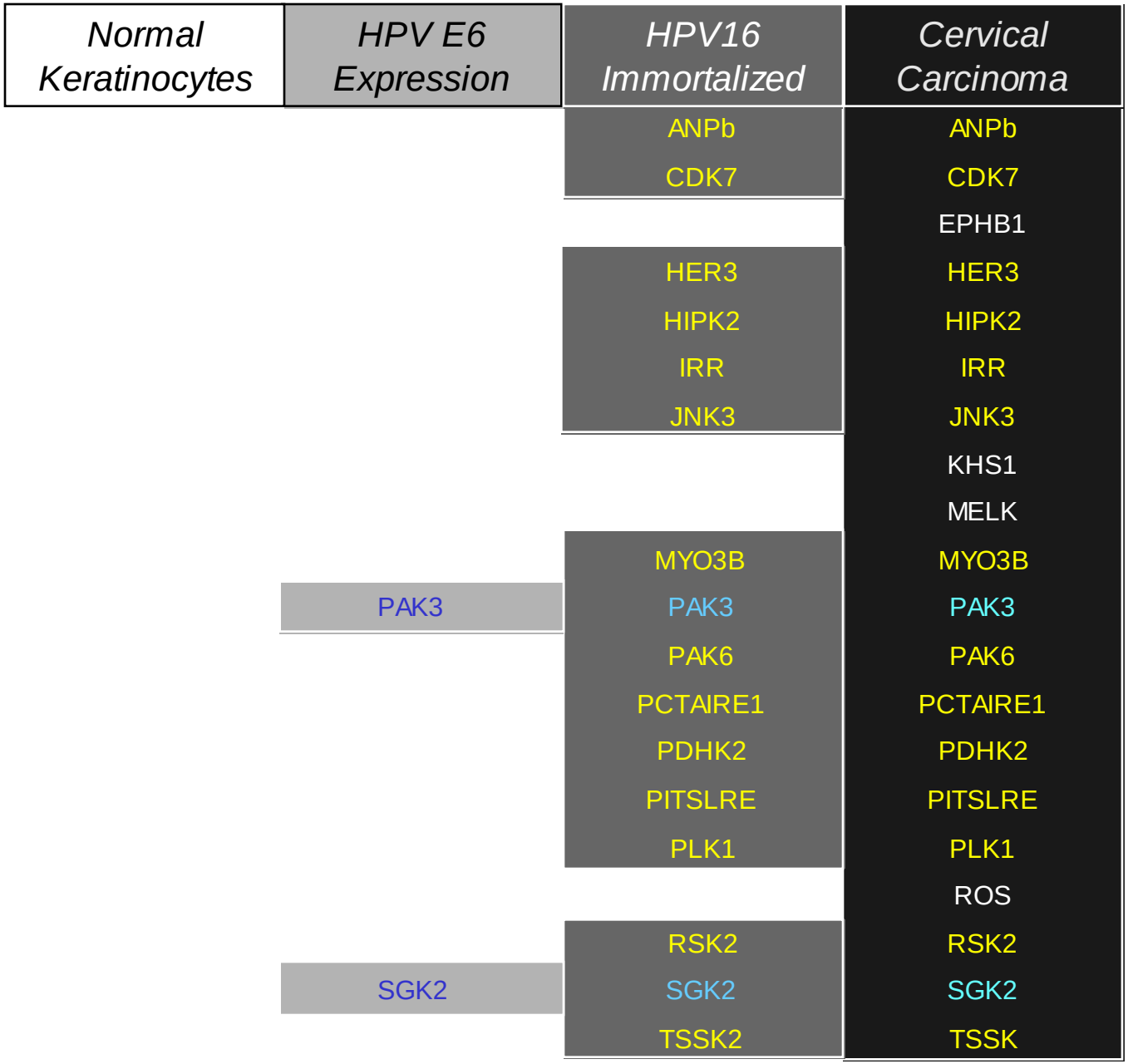
Normal Keratinocytes	*HPV E6 Expression*	*HPV16 Immortalized*	*Cervical Carcinoma*

"100 Hits" Kinase shRNA library

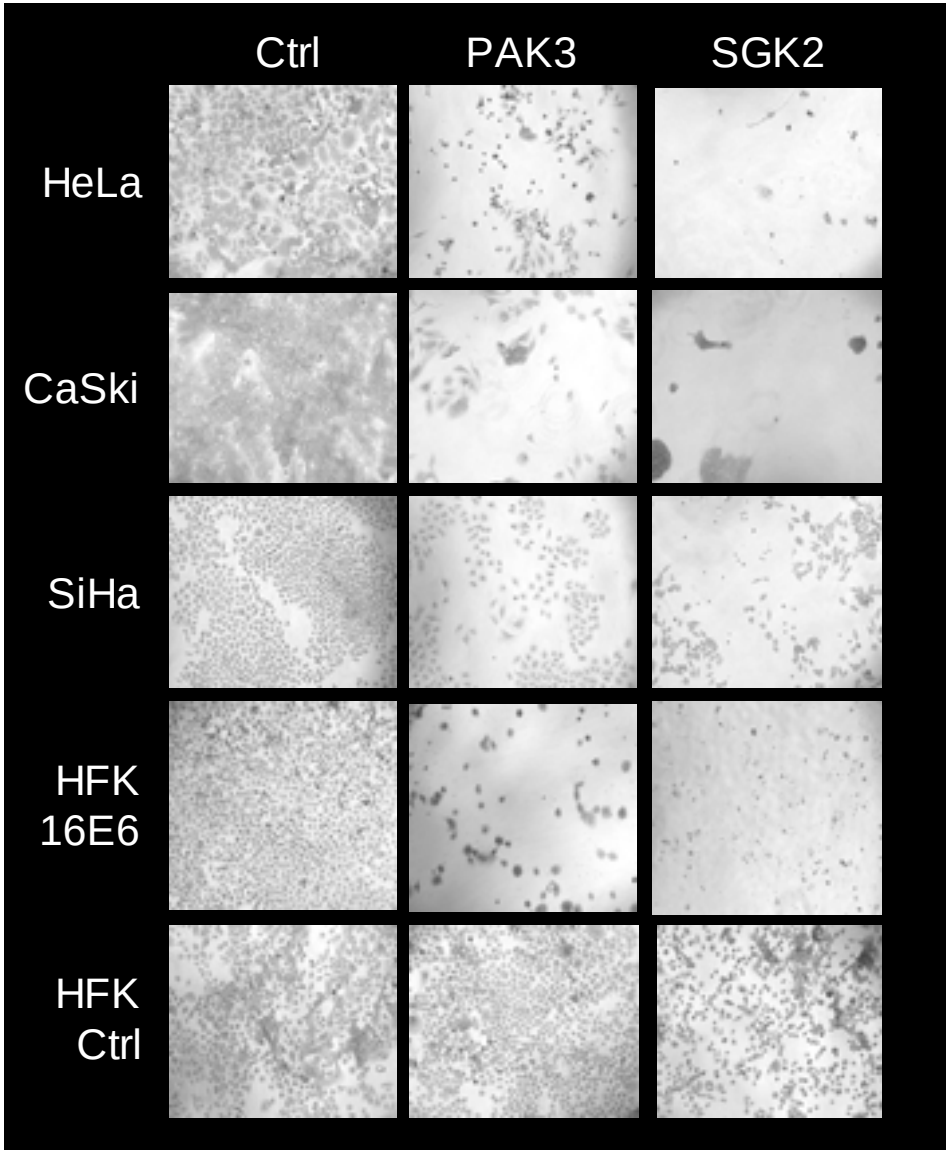
Targets a subset of 80 kinases that potently kill a variety of cancer cell lines

Identify essential kinases for each cell population




Normal Keratinocytes
HPV E6 Expression
HPV16 Immortalized
Cervical Carcinoma
ANPb
CDK7
EPHB1
HER3
HIPK2
IRR
JNK3
KHS1
MELK
MYO3B
PAK3
PAK6
PCTAIRE1
PDHK2
PITSLRE
PLK1
ROS
RSK2
SGK2
TSSK2
ANPb
CDK7
EPHB1
HER3
HIPK2
IRR
JNK3
KHS1
MELK
MYO3B
PAK3
PAK6
PCTAIRE1
PDHK2
PITSLRE
PLK1
ROS
RSK2
SGK2
TSSK

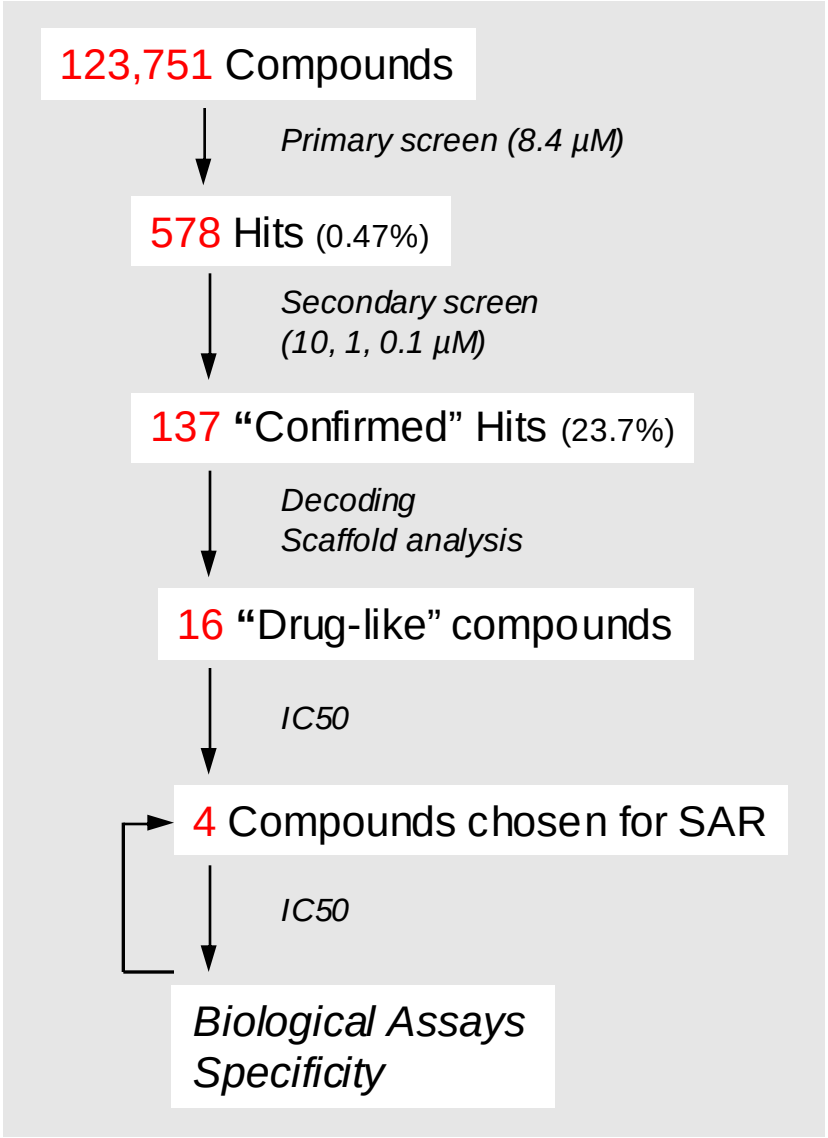
PAK3 and SGK2 become essential upon HPV E6 expression



Baldwin, Grueneberg

PAK3 Inhibitor Screen

Collaboration with Greg Cuny/Jun Xian (PCDD)



Karin Hellner

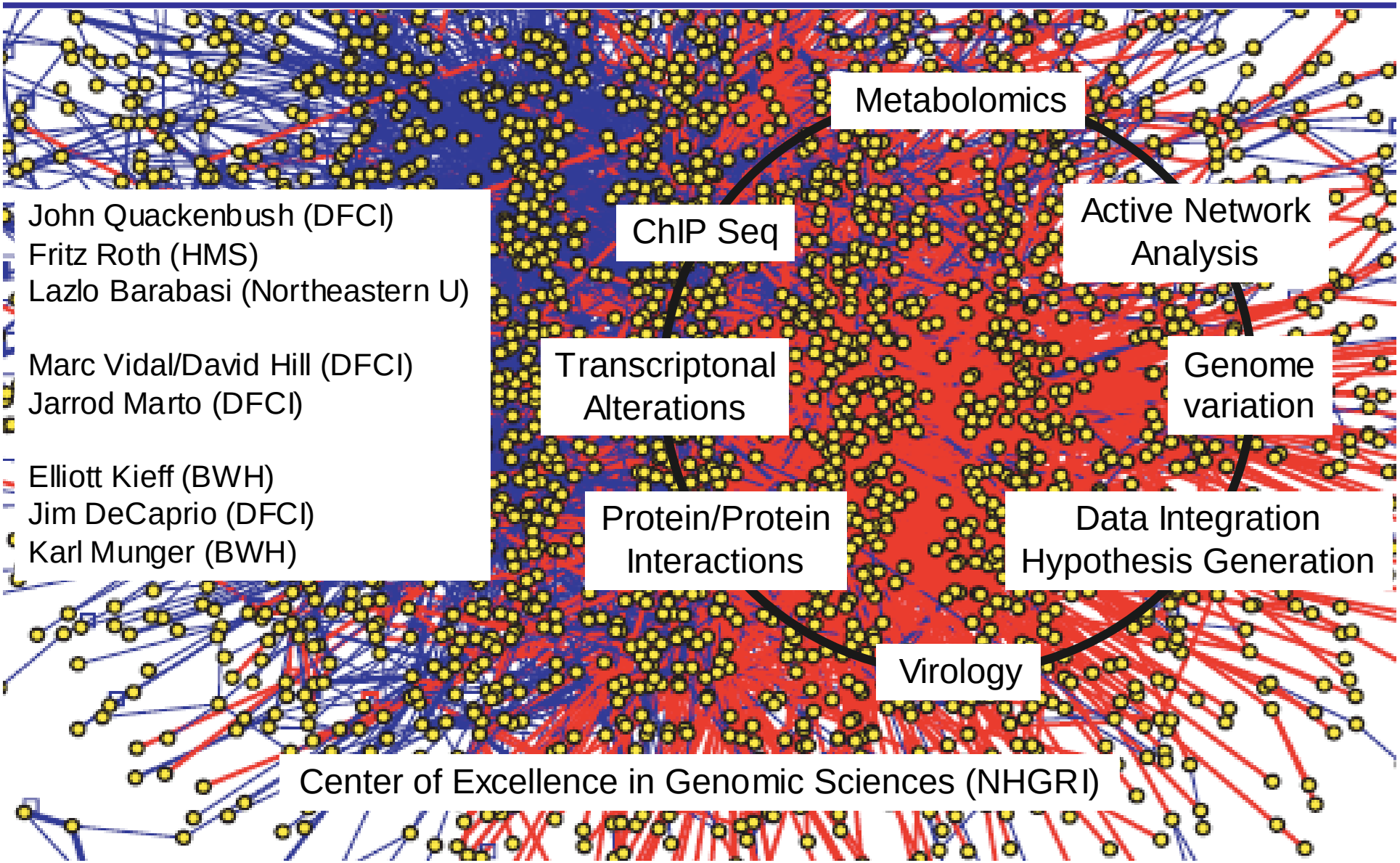
Identification of therapeutic targets

"Unbiased" approach

"You see what you can study"

Systems Biology

An integrative approach to identify cellular network perturbations induced by viral oncoprotein expression



Munger Lab

Alyce Chen
Sebastian Darr
Miranda Grace
Karin Hellner
Sergio Ita
Molly McLaughlin-Drubin
Jennifer Spangle
Yueyang Yu

Amy Baldwin
(St George's University, Grenada)
Kyung Won Huh
(LSUHSC, New Orleans, LA)

Ed Harlow (HMS)
Dorre Grueneberg (HMS)
The RNAi Consortium (Broad)

Marshall Posner (DFCI)
Jochen Lorch (DFCI)

Greg Cuny (PCDD)
Jun Xian (PCDD)

Marc Vidal (DFCI)

Chris Crum (BWH)

NIH
CA 066980
CA 081135
CA 141583
DE 015302
HG 004233

Lessons and Take Home Messages

- Virus associates cancers offer unique opportunities for prevention, diagnosis and therapy
- Some mechanistic insights obtained with HPV-associated cancers should be generally applicable to non-virus associated human cancers
- Unbiased genetic screens and integrative, system biology based approaches will define novel therapeutic targets