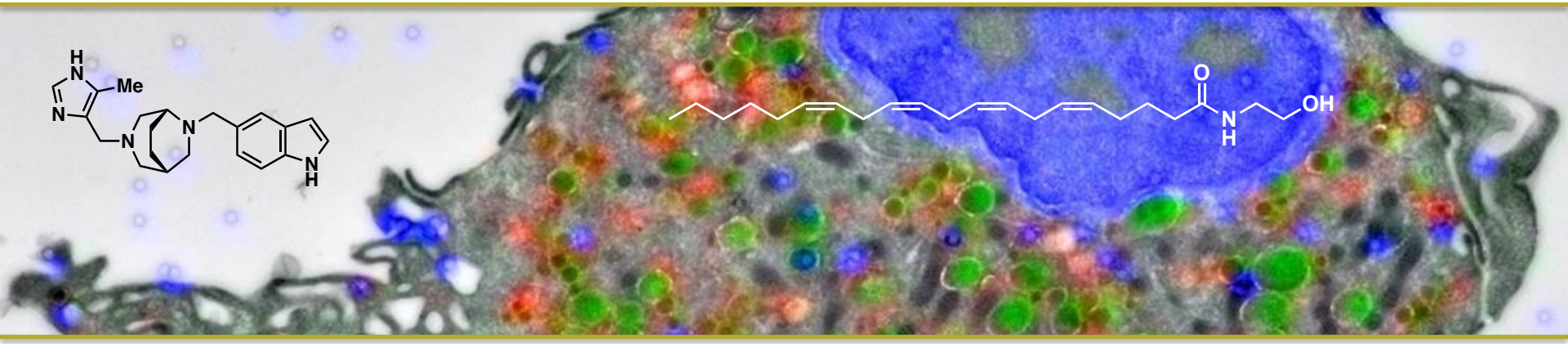
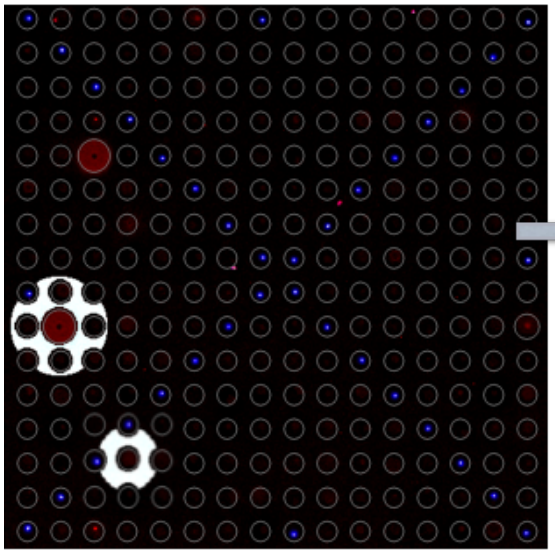


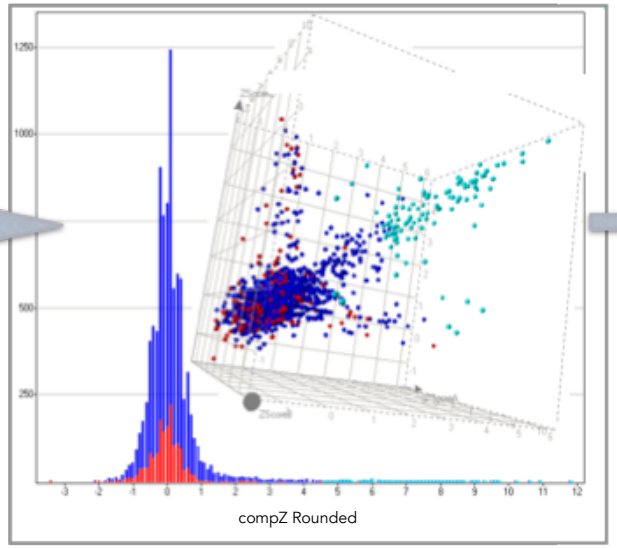
L5 – A Probe Discovery Vignette

February 17, 2022

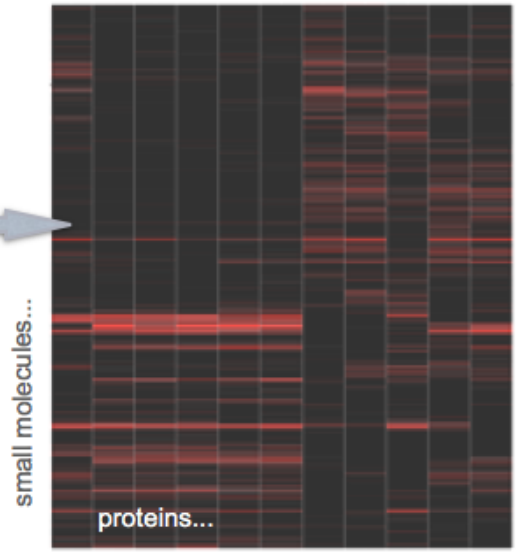
From hits to probes –> validation



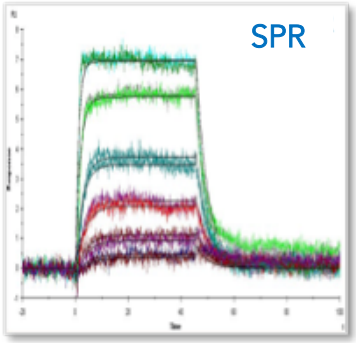
fluorescent features reveal putative
TDP43-ligand interactions

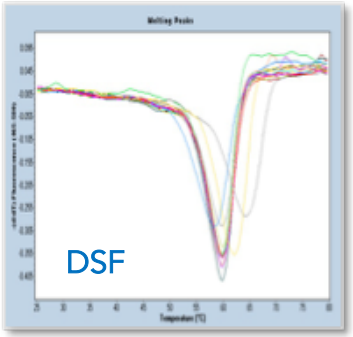


compute composite Z-scores, 'hit' calls



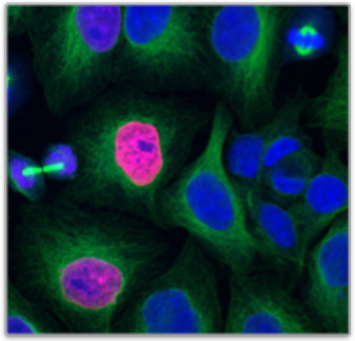
specificity analysis across proteins

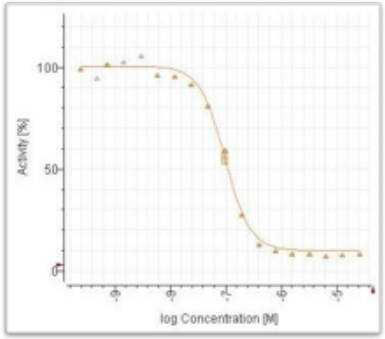




secondary, quantitative binding assays

+

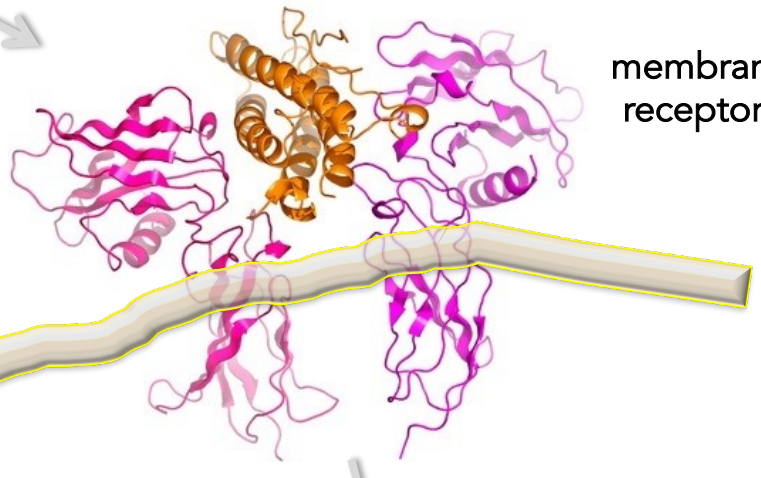




functional assays (e.g. cellular, biochemical)

extracellular factors

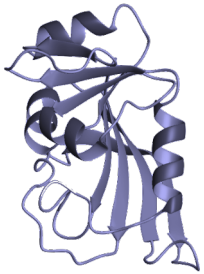
clotting factors
(DVT, hemophilia)
one of the best drugged classes!



membrane receptors

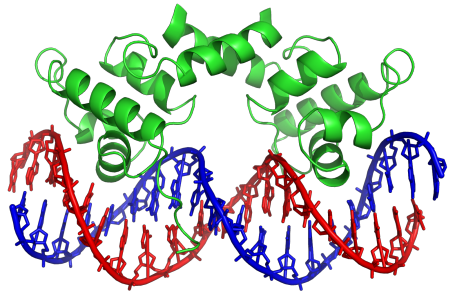
ion channels
receptor kinases
(oncology, neurology, mood disorders, inflammation)

cellular response



intracellular signaling proteins

kinases
metabolic enzymes
(many diseases)



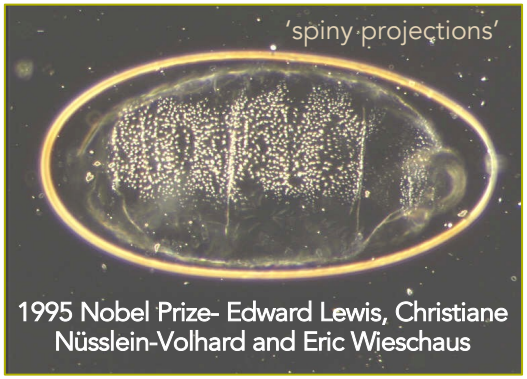
transcriptional regulators

nuclear hormone receptors
(many diseases)

Sonic hedgehog protein

important role in development including limb and brain development

1978- Embryogenesis
Mutational Genetic Screen
mutant hedgehog drosophila larva



mutations in Shh are linked with
Holoproscencephaly (HPE)

'cyclopia'

M. Muenke, *Seminars in Developmental Biology* Vol. 5, 293-301, 1994

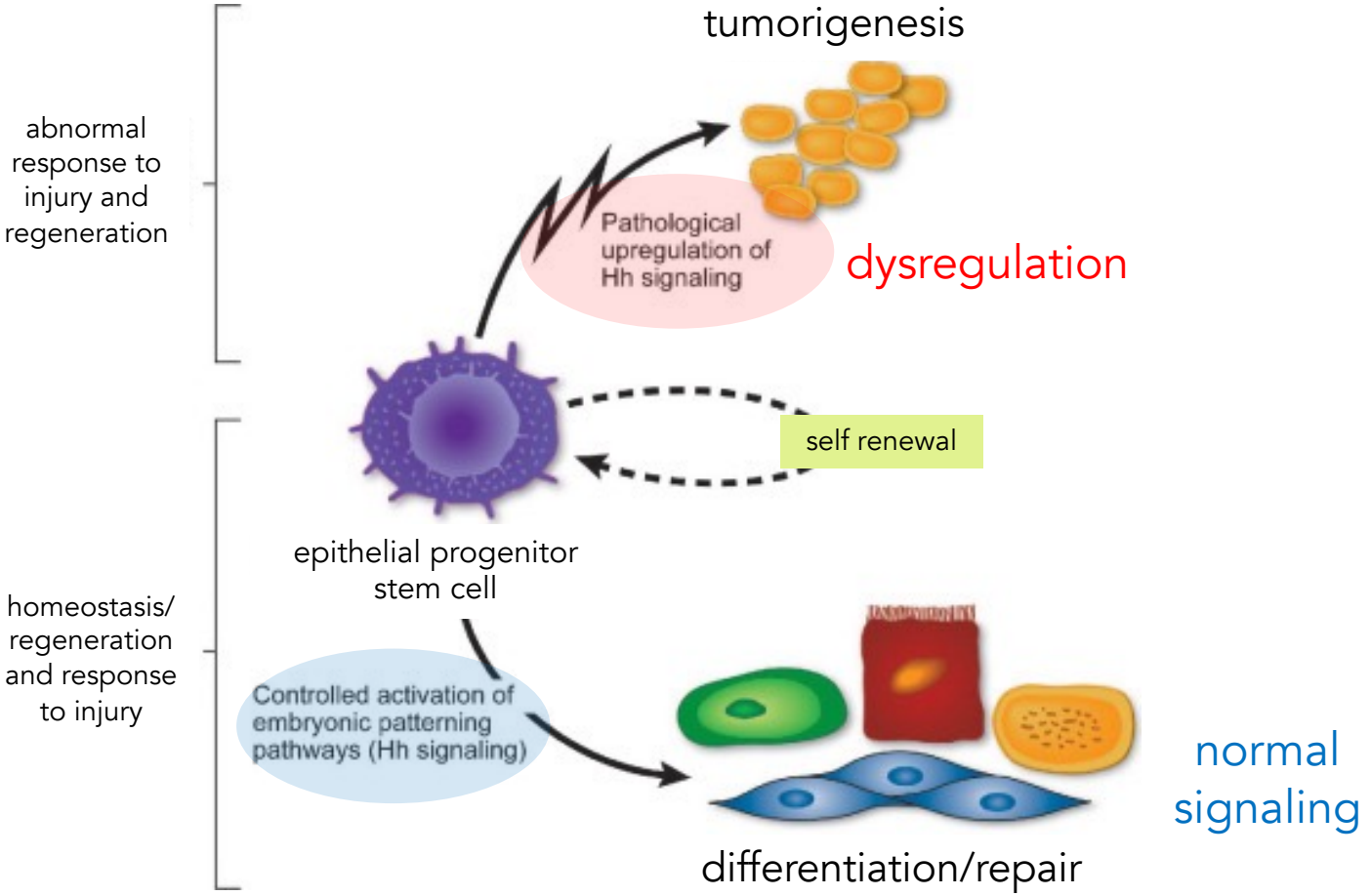
3 Hh genes

Desert and Indian
(Dhh and Ihh)

Sonic
(Shh)

Hedgehog signaling goes beyond embryogenesis

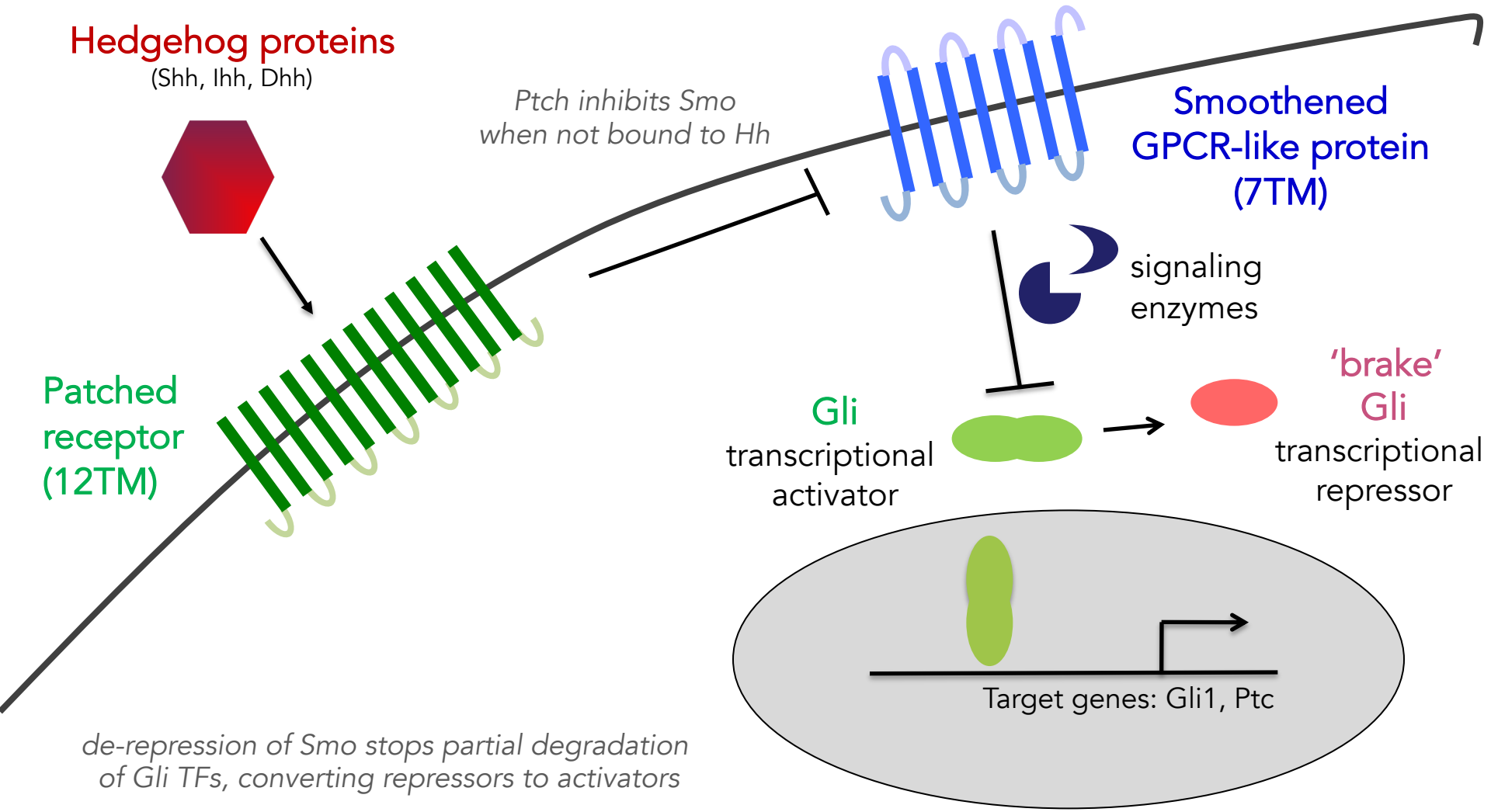
development, differentiation, and disease



Hh signaling pathway involved in embryogenesis plays a critical role in the maintenance of stem cells in adult life and cellular responses to injury

Hedgehog proteins 'de-repress' Smoothened

Hh-Ptch binding interaction activates Gli-driven transcription

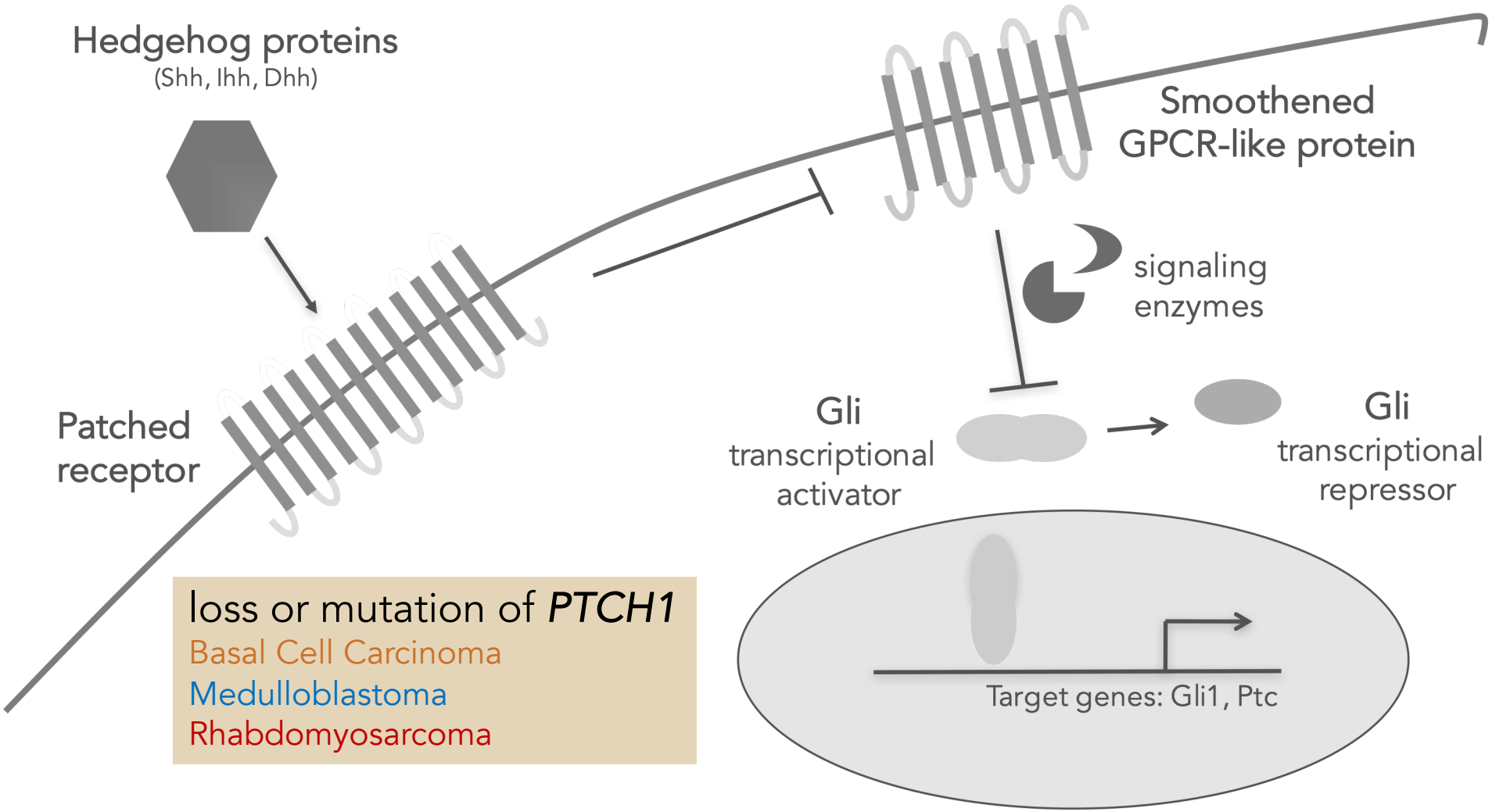


overexpression of *SHH*

Pancreatic Cancer (70%) Prostate Cancer
Gastric Cancer Lung Cancer
Medulloblastoma Ovarian Cancer

activating mutations in *SMO*

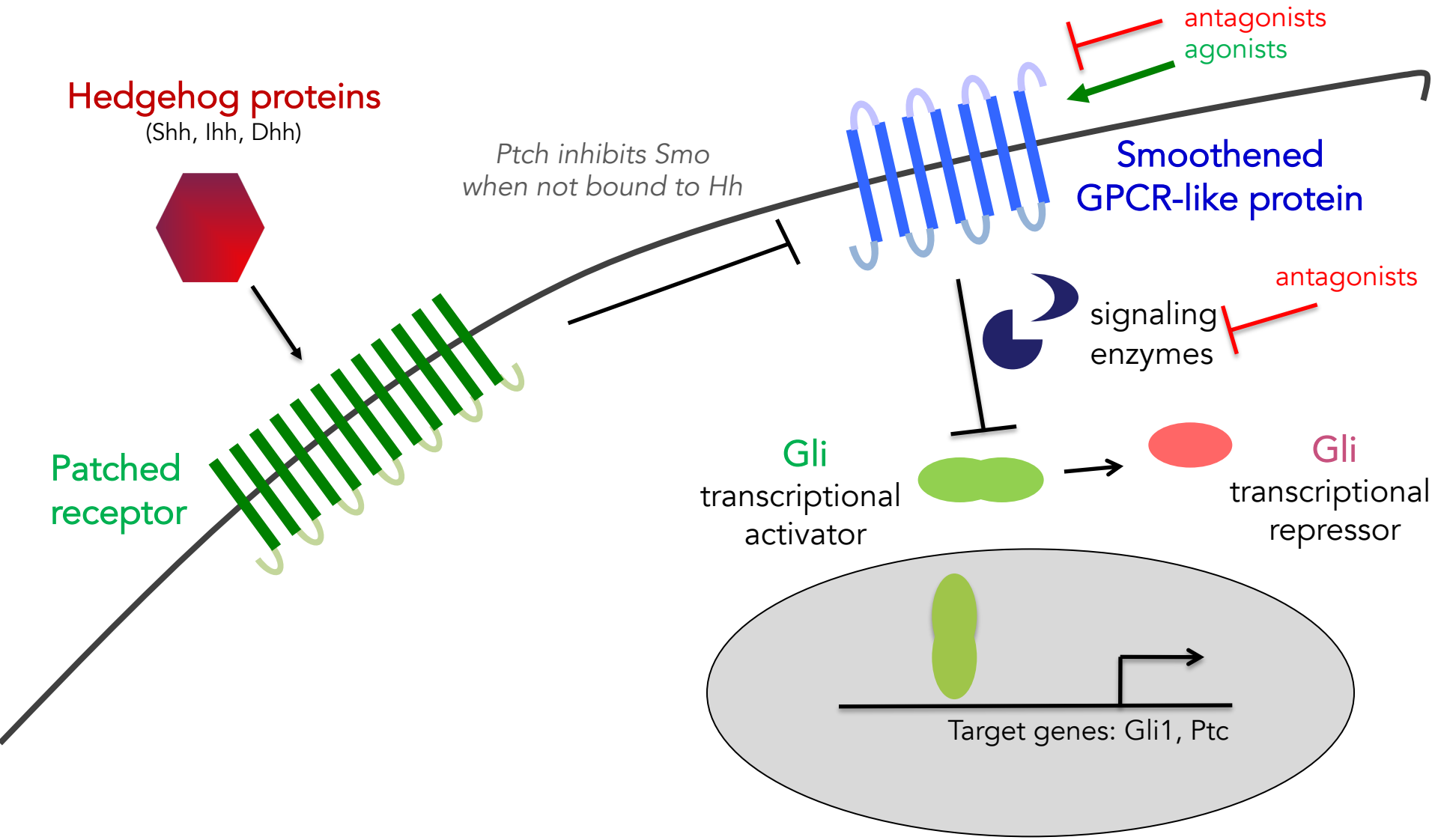
Basal Cell Carcinoma
Ovarian Cancer



loss or mutation of *PTCH1*

Basal Cell Carcinoma
Medulloblastoma
Rhabdomyosarcoma

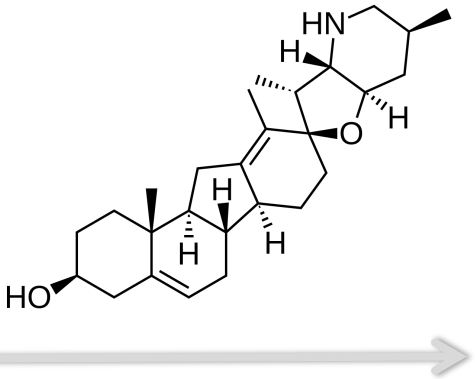
Drugs targeting Hedgehog pathway



Cyclopamine

Smo antagonist and Hh pathway inhibitor

Veratrum californicum
wild corn lily



11-yr investigation
By US Dept of Agriculture

cyclopic lamb born of
a sheep that ate corn lily
(Idaho farm, 1957)

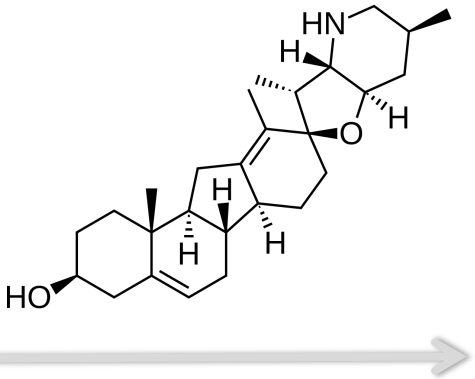
Beachy & Chen Labs (Stanford): Cyclopamine inhibits Hh signaling by influencing the balance of active and inactive Smoothened protein

How did they arrive at this conclusion?

Cyclopamine

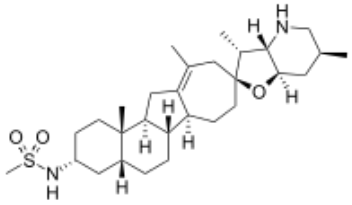
lead for development of anti-cancer agents

Veratrum californicum
wild corn lily

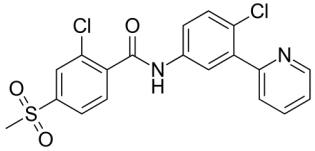


cyclopic lamb born of
a sheep that ate corn lily
(Idaho farm, 1957)

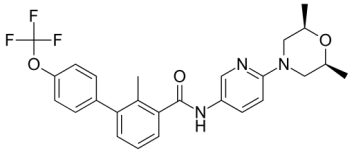
Adult cancers - basal cell carcinoma, medulloblastoma, prostate, breast, pancreas



saridegib

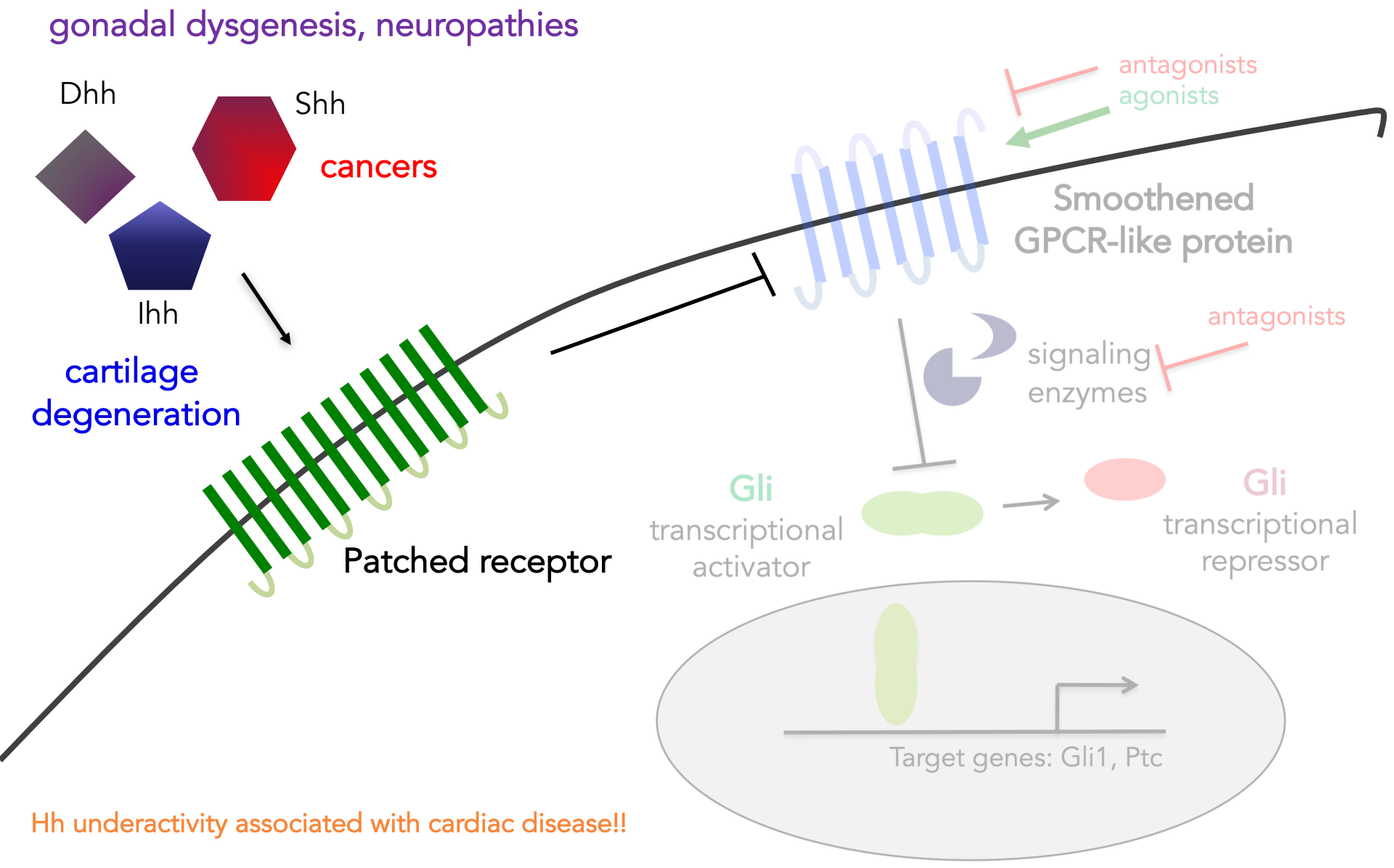


vismodegib



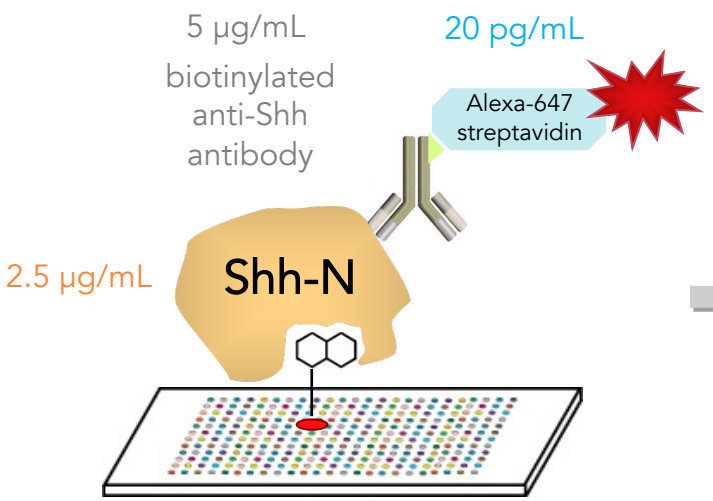
sonidegib

Selective targeting of Hh signaling upstream of Smo



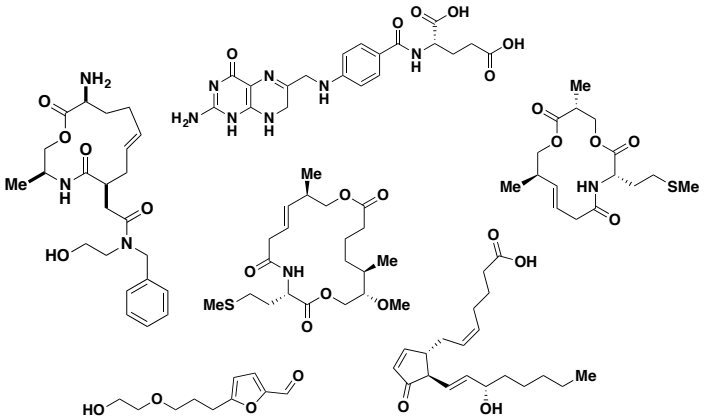
Hh underactivity associated with cardiac disease!!

SMM assay: 20 kDa Shh N-terminal fragment



~10,000 printed compounds
(small molecule microarray)

19 SMM hits



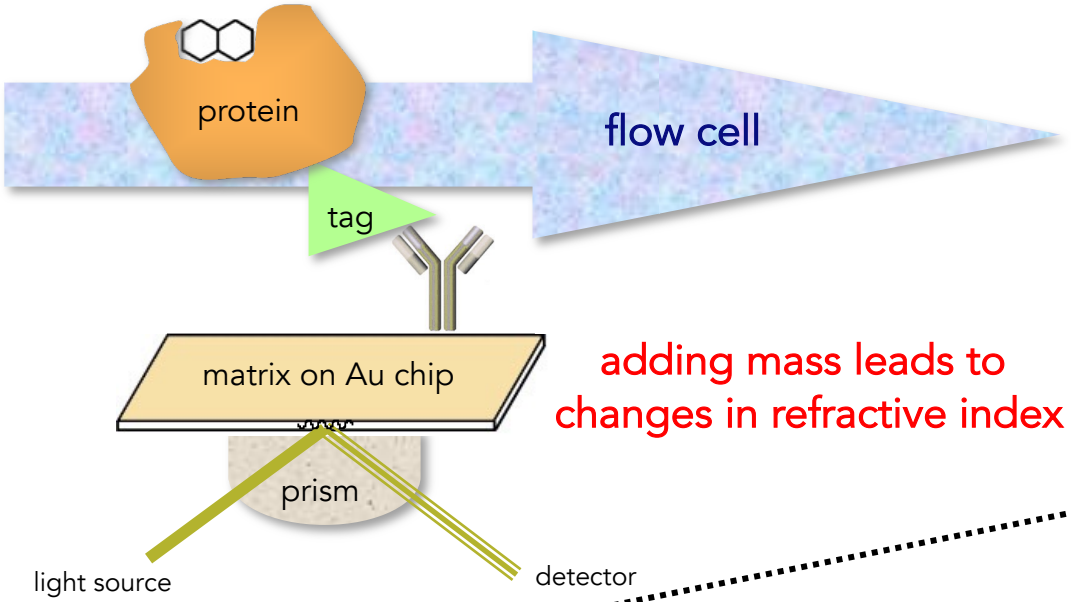
Angela, Broad Fellow

Lee Peng, MGH

Ben Stanton, Harvard

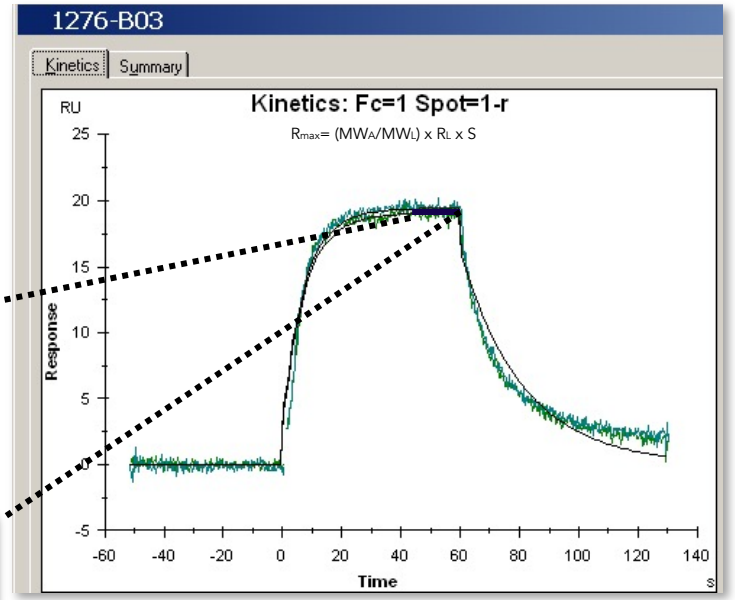
Validating assay positives in secondary binding assays

'mass sensing' by Surface Plasmon Resonance (SPR)

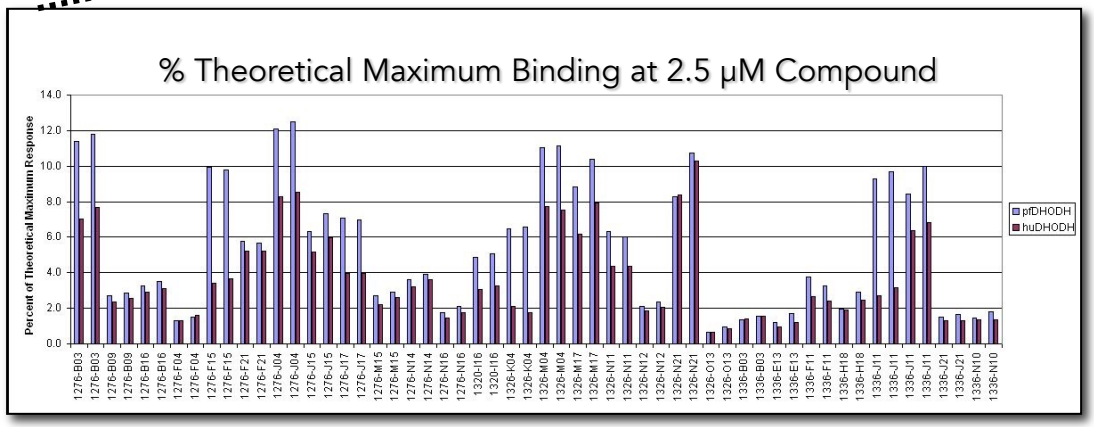


adding mass leads to changes in refractive index

kinetic assay – real time measurement

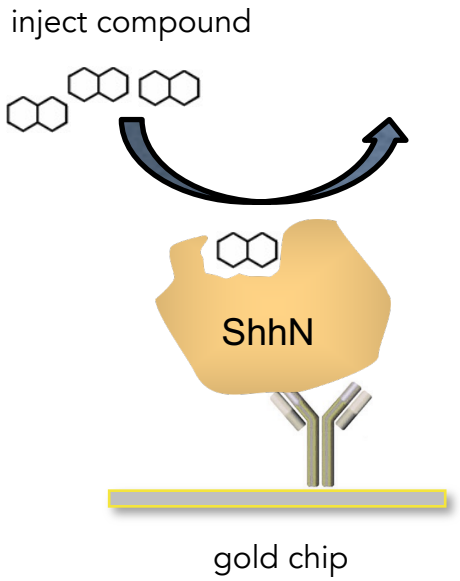


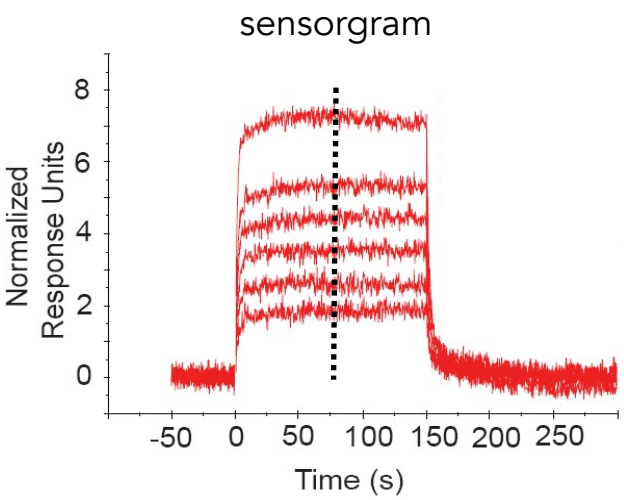
76% of SMM interactions retest by SPR

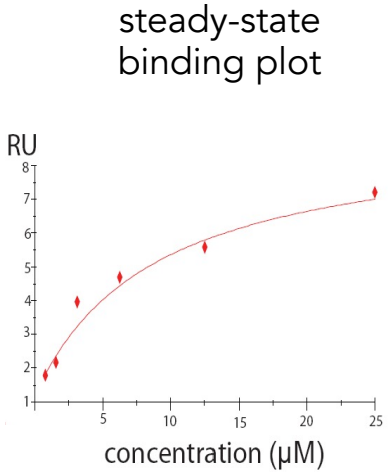


For Video on SPR, go to:
https://www.youtube.com/watch?v=o8d46ueAwXI

SPR experiments for Shh SMM hits







K_D = 9 μM

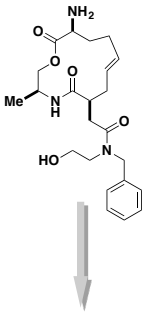
- reverses orientation from primary assay
- measures binding between immobilized protein and compounds injected in solution
- kinetic measurements
- ranking assays (k_{on} vs. $k_{off,}$ % Ru_{max})
- compound affinity characterization

Measuring *GLI*-dependent transcriptional activity

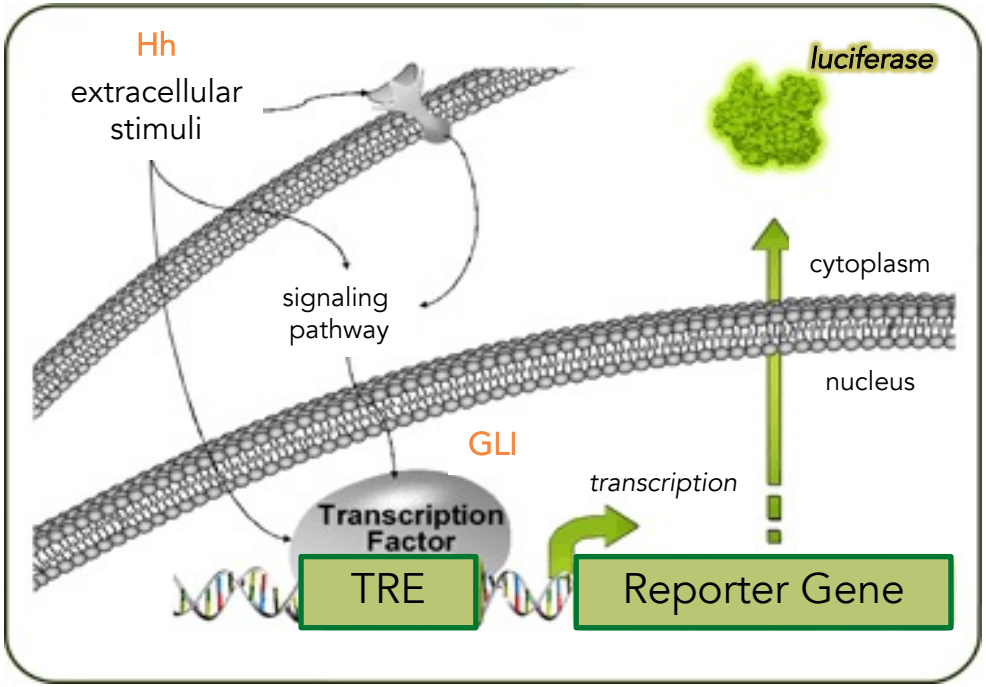
quantitative assay for hedgehog signaling

1

K_D = 9 μM



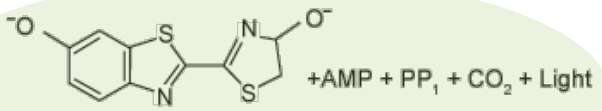
NIH/3T3 cell line transfected with GLI-responsive reporter assay vector



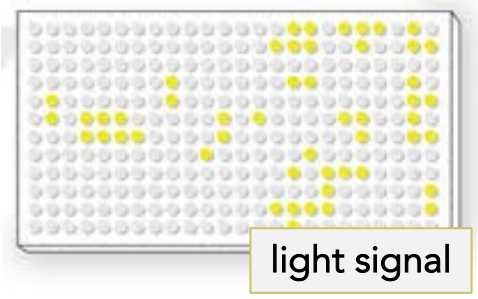
luciferase reaction

Firefly Luciferin

Firefly Luciferase **Mg^{2+}**



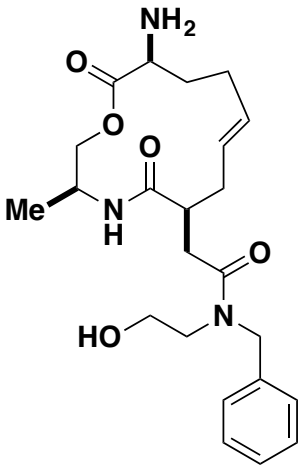
Oxyluciferin



light signal

Measuring *GLI*-dependent transcriptional activity

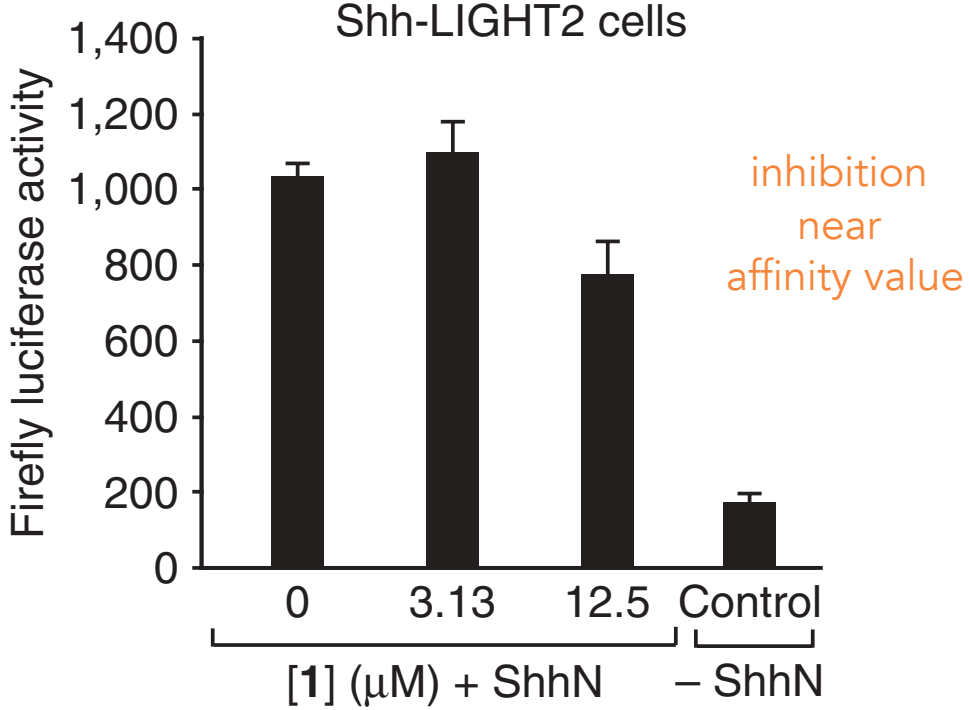
SMM hit modulates transcriptional output in preliminary experiment



1

K_D = 9 μM

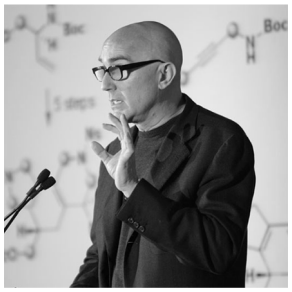
reporter assay



inhibition near affinity value

each value represents 5 technical replicates
error bars denote standard deviation

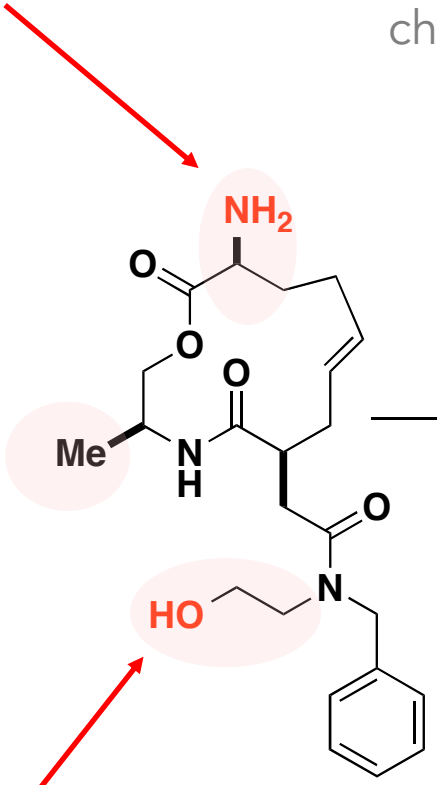
Stuart Schreiber, Harvard



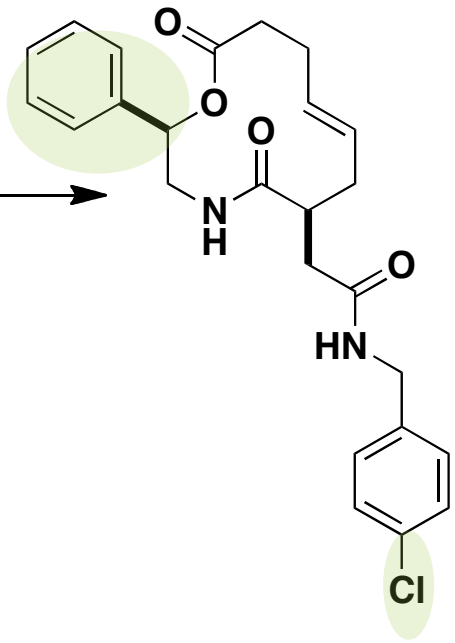
Hit to probe

chemical editing

site of attachment to SMM



18 variants



site of attachment to SMM

K_D = 9 μM

K_D = 3 μM

improved binding affinity

Remove – ethanolamine, methyl, amine and carbon
Add – phenyl, chloro groups

Doctor Ivo "Eggman" Robotnik

Robotnikinin

Shh binder and antagonist

nature chemical biology

A small molecule that binds Hedgehog and blocks its signaling in human cells

Benjamin Z Stanton[1,2,7], Lee F Peng[1–3,7], Nicole Maloof[1], Kazuo Nakai[2], Xiang Wang[1], Jay L Duffner[1], Kennedy M Taveras[1], Joel M Hyman[4], Sam W Lee[5], Angela N Koehler[1], James K Chen[4], Julia L Fox[6], Anna Mandinova[5] & Stuart L Schreiber[1,2]

Small-molecule inhibition of extracellular proteins that activate membrane receptors has proven to be extremely challenging. Diversity-oriented synthesis and small-molecule microarrays enabled the discovery of robotnikinin, a small molecule that binds the extracellular Sonic hedgehog (Shh) protein and blocks Shh signaling in cell lines, human primary keratinocytes and a synthetic model of human skin. Shh pathway activity is rescued by small-molecule agonists of Smoothened, which functions immediately downstream of the Shh receptor Patched.

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Sonic the Hedgehog

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Celebrate Math Awareness Month — Shop Now

Guest Blog

In Silico et Vivo: When Life Science Draws Inspiration from Video Games

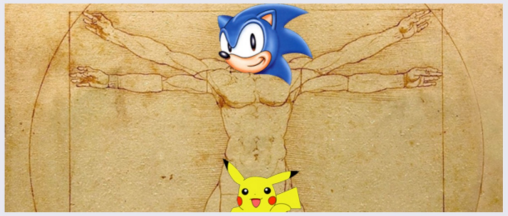
By Jon Chang on October 12, 2012

You Got Your Genetics In My Videogames

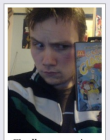
We here at GameCola's crack news team are dedicated to searching far and wide for videogame news, but today I'll be daring to traverse the very time-space continuum to bring you news from several year

Contributed by Christian Porter on April 8th, 2011 in News Posts

With content involving Christian Porter, Dr. Eggman, Dr. Robotnik, genetics, News, Pikachu, Pikachurin, Pokémon, retinitis pigmentosa, Robotnikinin, science, Sonic Hedgehog Homolog, Sonic the Hedgehog



We here at GameCola's crack news team are dedicated to searching far and wide for videogame news, but today I'll be daring to traverse the very time-space continuum to bring you news from several years in the past—**just for you, valued reader!**



Finally, my precious Mega Drive, we are one. My plans are complete.

Normally I expect to see references to videogames in *Game Informer*, not the science journal *Nature*, but it looks like gamer geneticists have been tasked with naming the mammalian gene responsible for the growth of fingers and toes, stem cell division, and the organization of the brain. It's name? Sonic Hedgehog Homolog. Yes, this means that Sonic the Hedgehog is inside of all of our genes, and, judging based on his love of the azure rodent, is now all over the inside of an overly-excited Matt Jonas's underwear upon hearing this information.

Additionally, it turns out that Harvard researchers created an equally hilarious inhibitor to counteract Sonic Hedgehog Homolo: Robotnikinin.

RETROIST

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Retroist Sonic the Hedgehog Podcast

By Retroist | April 30, 2010 | 2 Comments

RETROIST PODCAST

SONIC THE HEDGEHOG

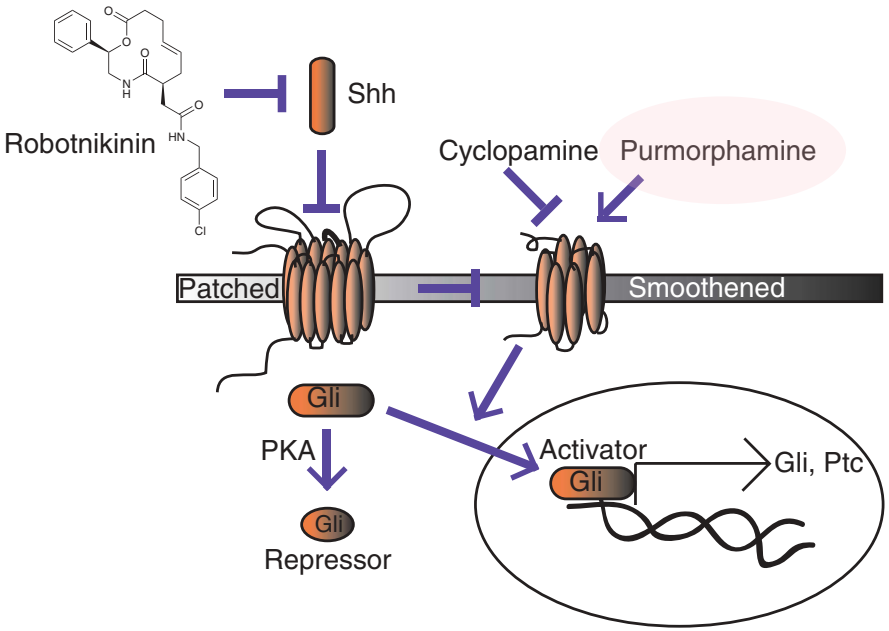
9 February 2013 / 1,237 notes / 6 comments

Share Trivia https://tmblr.co/ZqM2Ivdid7

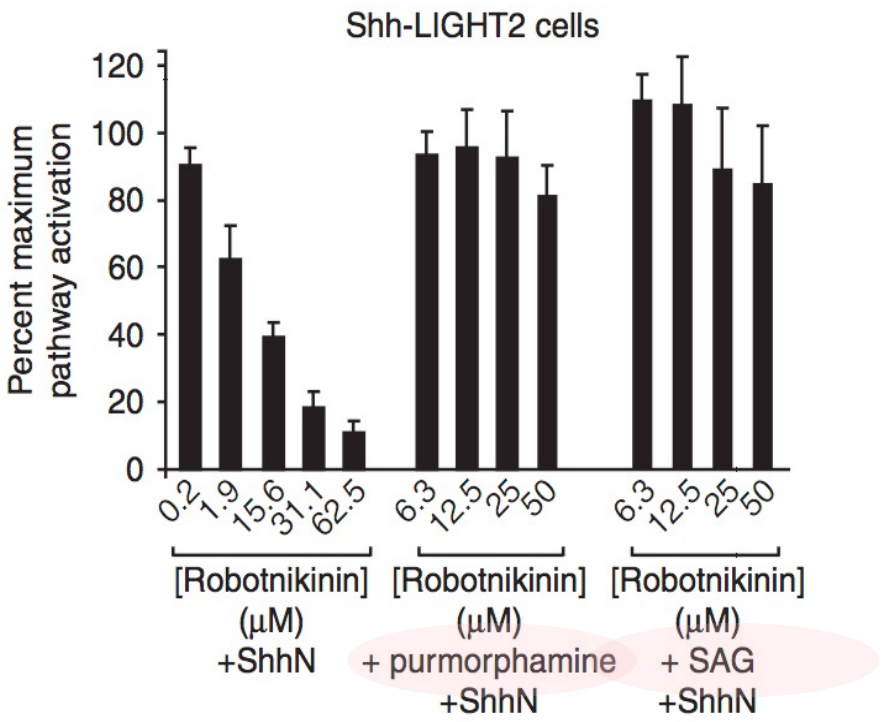
Sonic the Hedgehog.

http://www.tssznews.com/2009/02/21/sonic-hedgehog-vs-robotnikinin/

Gli inhibition by Robotnikinin is rescued by a Smoothened agonist

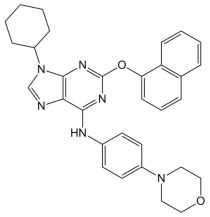


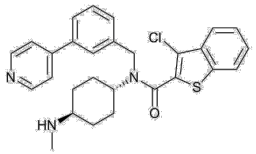
reporter assay



rescue experiments are common in systems biology

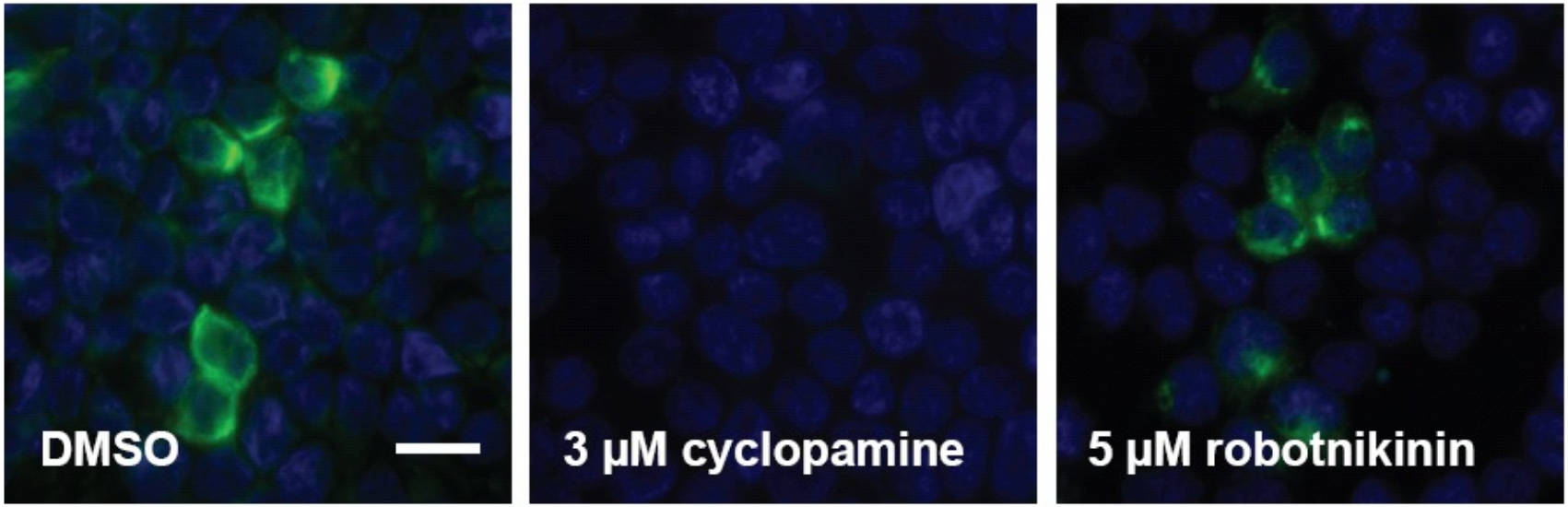
validate mechanistic hypotheses





Ligand competition assays to assess specificity

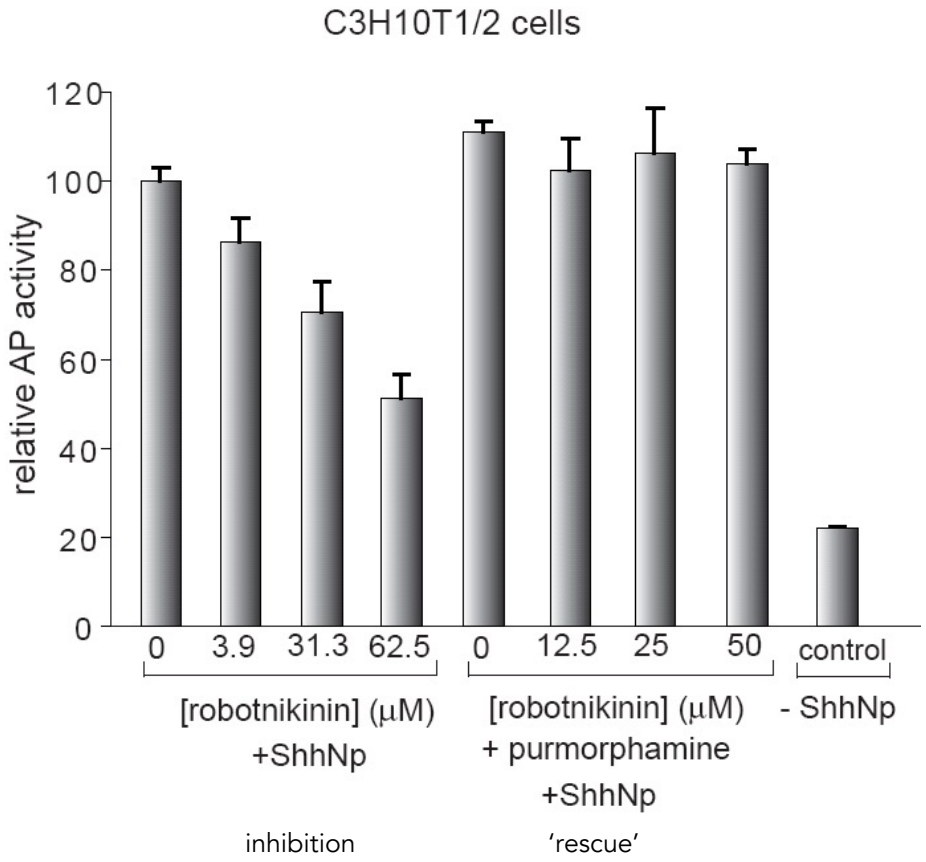
BODIPY-cyclopamine binds to Smoothened at cell surface



Smoothened-overexpressing human embryonic kidney cells

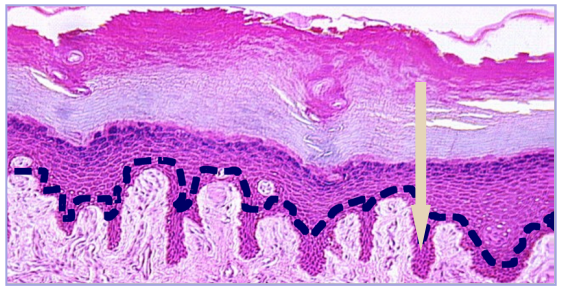
Conclusion: Robotnikinin does not compete with a labeled Smo ligand

Inhibition of stem cell differentiation



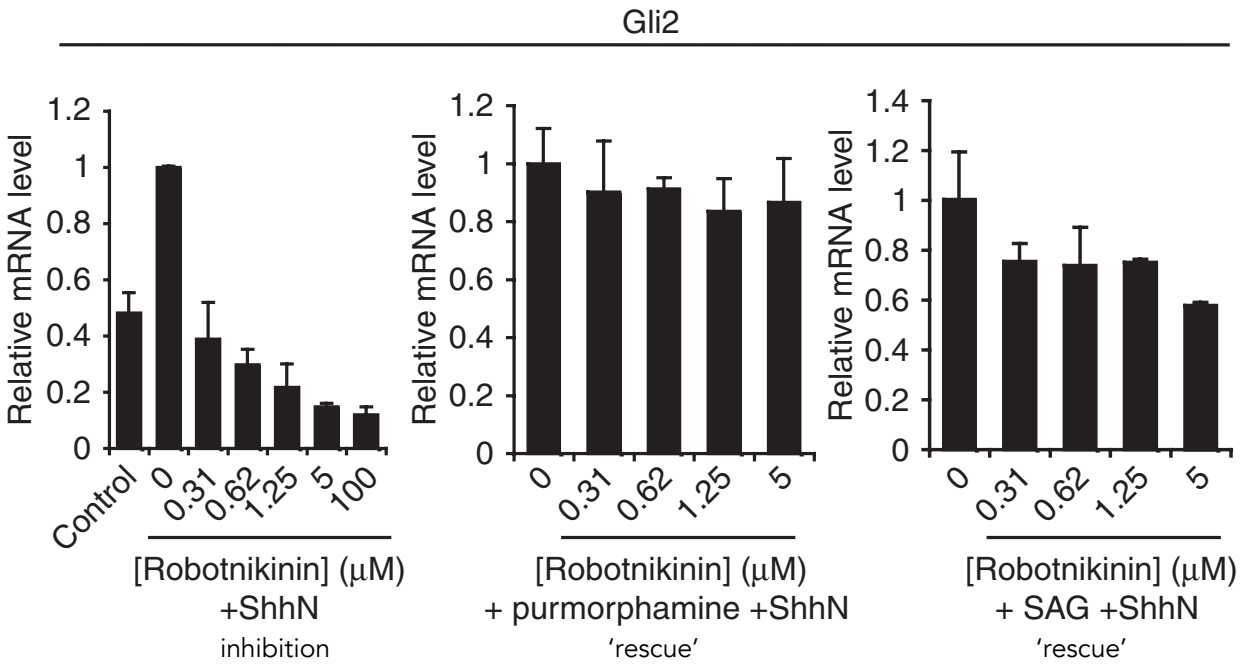
mouse mesenchymal stem cells differentiate into osteoblasts and upregulate alkaline phosphatase (AP) when stimulated with N-palmitoylated ShhN

Skin: Robotnikinin lowers levels of *GLI2* mRNA in primary human keratinocyte cells

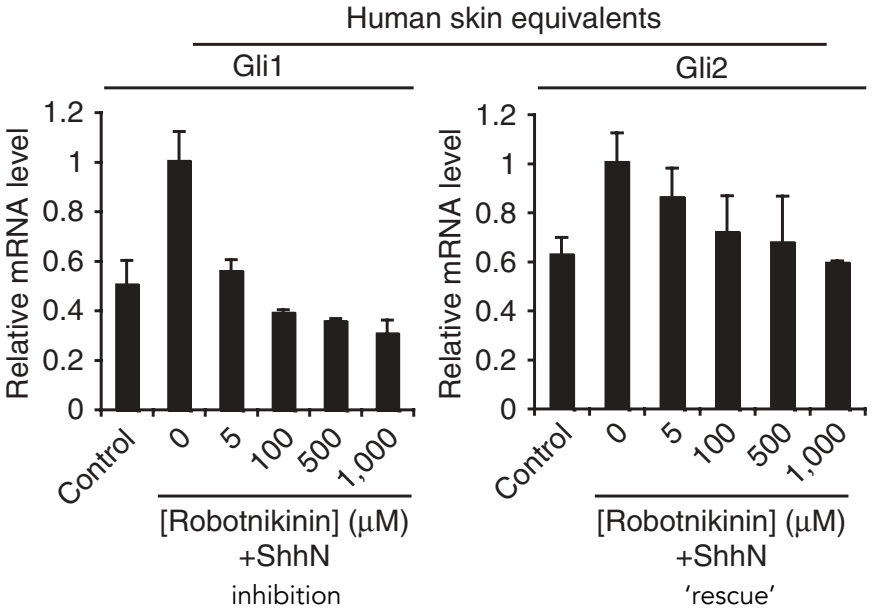


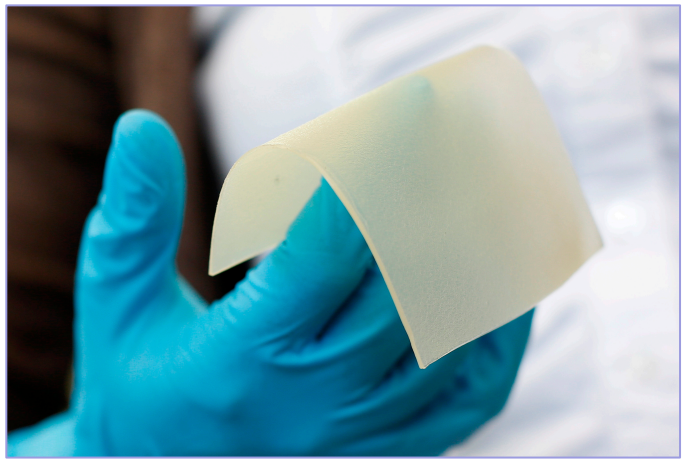
primary human keratinocytes
isolated from the basal cell layer

measure mRNA by quantitative
PCR after 30-hr treatments



Robotnikinin blocks lowers levels of *GLI1 and GLI2* mRNA in synthetic human skin





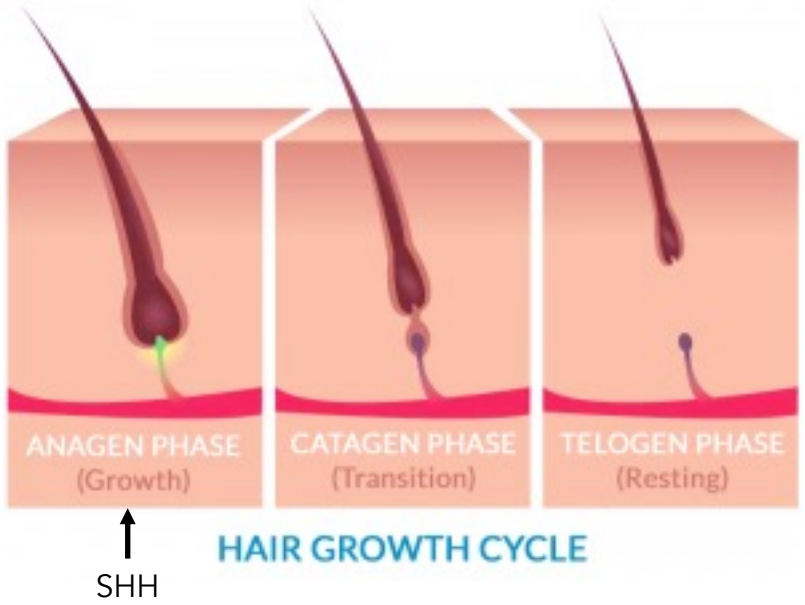
MGH synthetic human skin model:

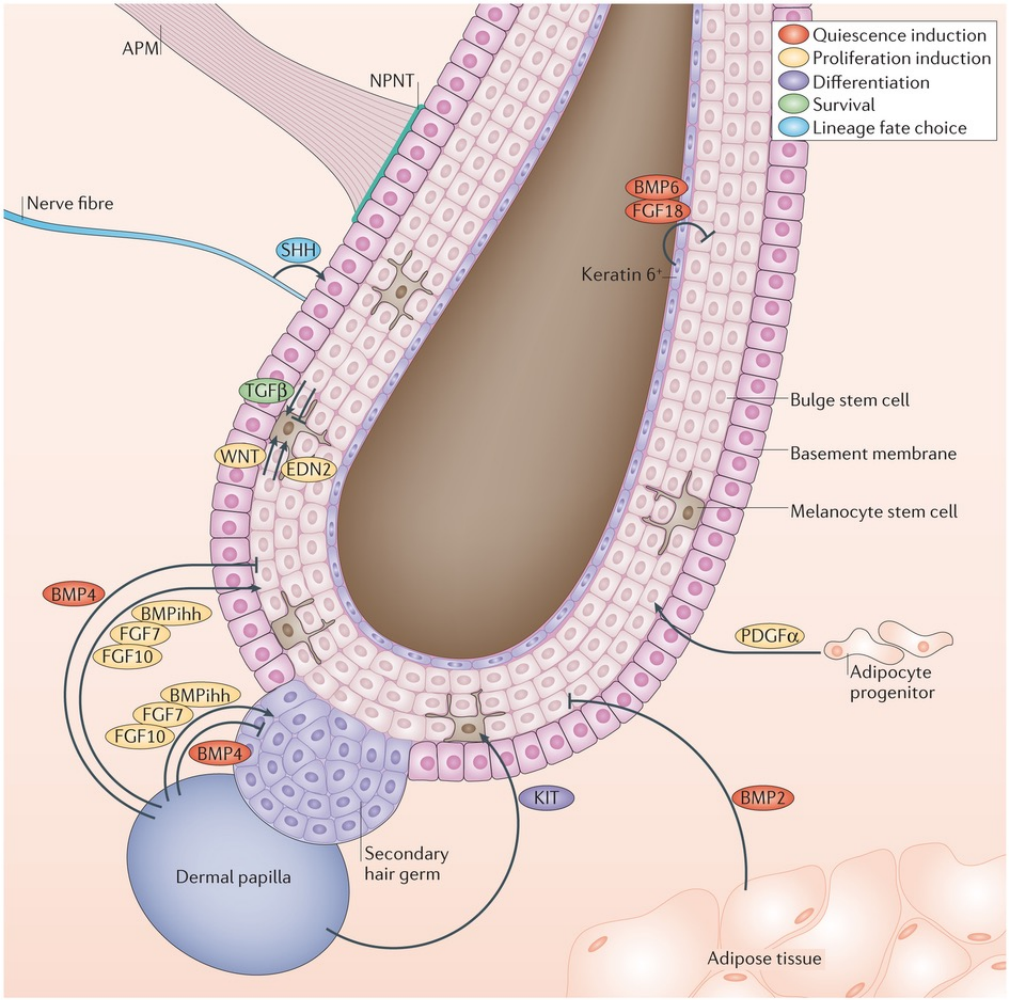
1. Extract dehydrated collagen matrix from skin grafts
2. Populate matrix with primary keratinocytes
3. Culture to form several dermal layers
4. Incubate with compound, analyze by qPCR and histology

Anna Mandinova, MGH

Structure-activity relationship
Syn-skin punches

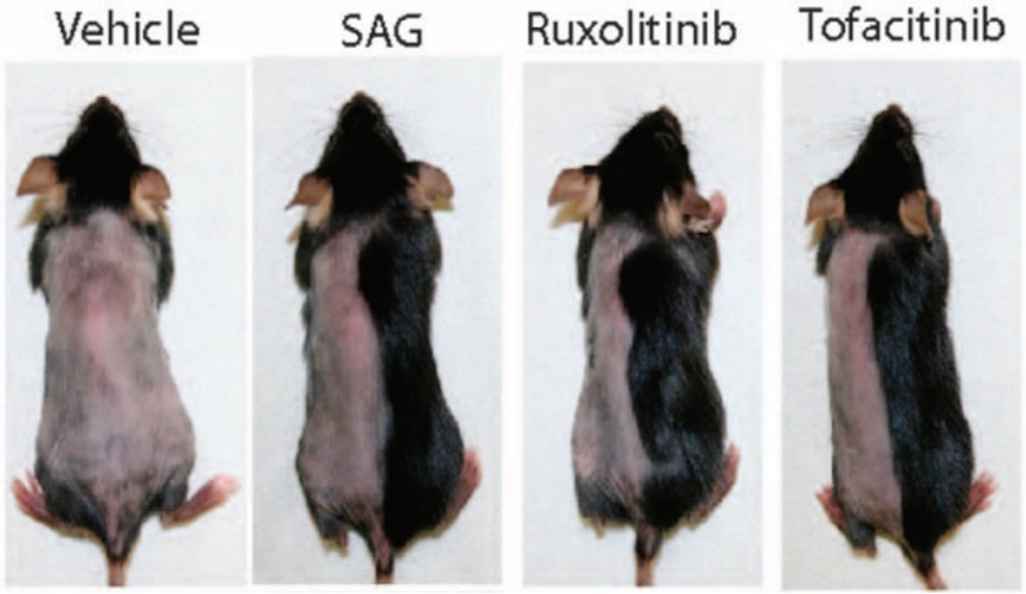
Shh and the hair follicle – a regulator of luscious locks





lower levels of Hh expression or signaling is associated with baldness

Exploring stimulation of Shh pathway as a way to promote hair growth



Seven-week-old wild-type mice were shaved and treated daily with either a topical application of vehicle control, sonic hedgehog agonist (SAG), 3% ruxolitinib (JAK1/2 inhibitor), or tofacitinib (JAK3 inhibitor). Skin was harvested at the indicated time points and stained with hematoxylin and eosin (H&E). Images of mice were taken at D21 of treatment. Harel et al. Sci. Adv. 2015

Smoothened agonists used in our rescue experiments

Robotnikinin inhibits hair growth *in vitro*

8 days post depilation

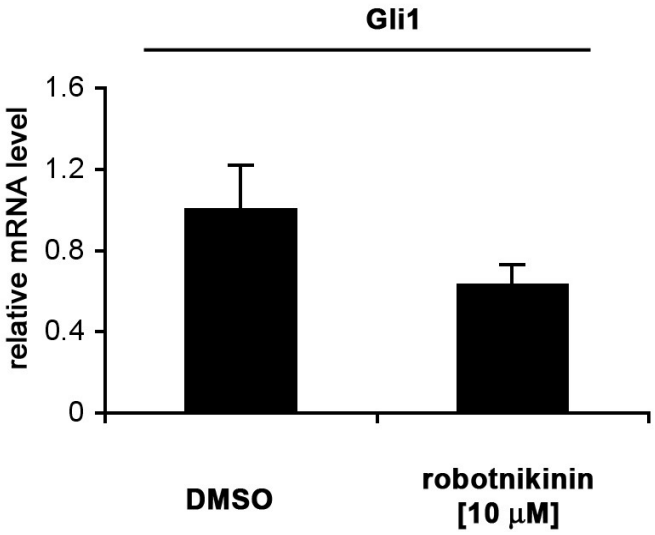
10 uM robotnikinin DMSO

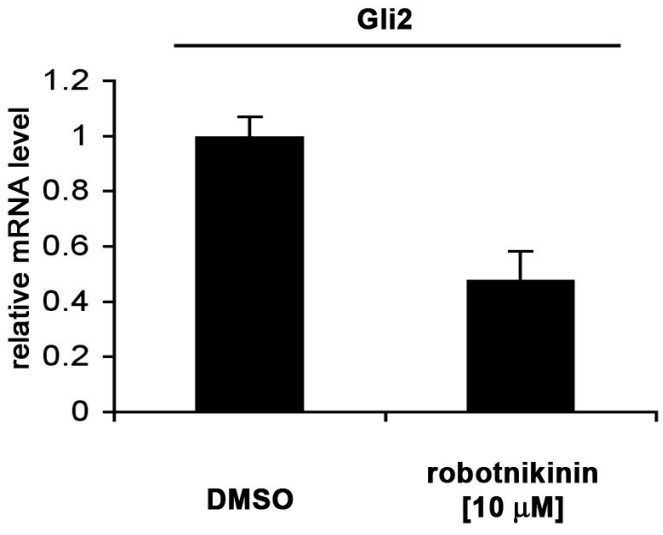
12 days post depilation

10 uM robotnikinin DMSO

Robotnikinin causes hair follicles to fail anagen phase entry

12 days post depilation

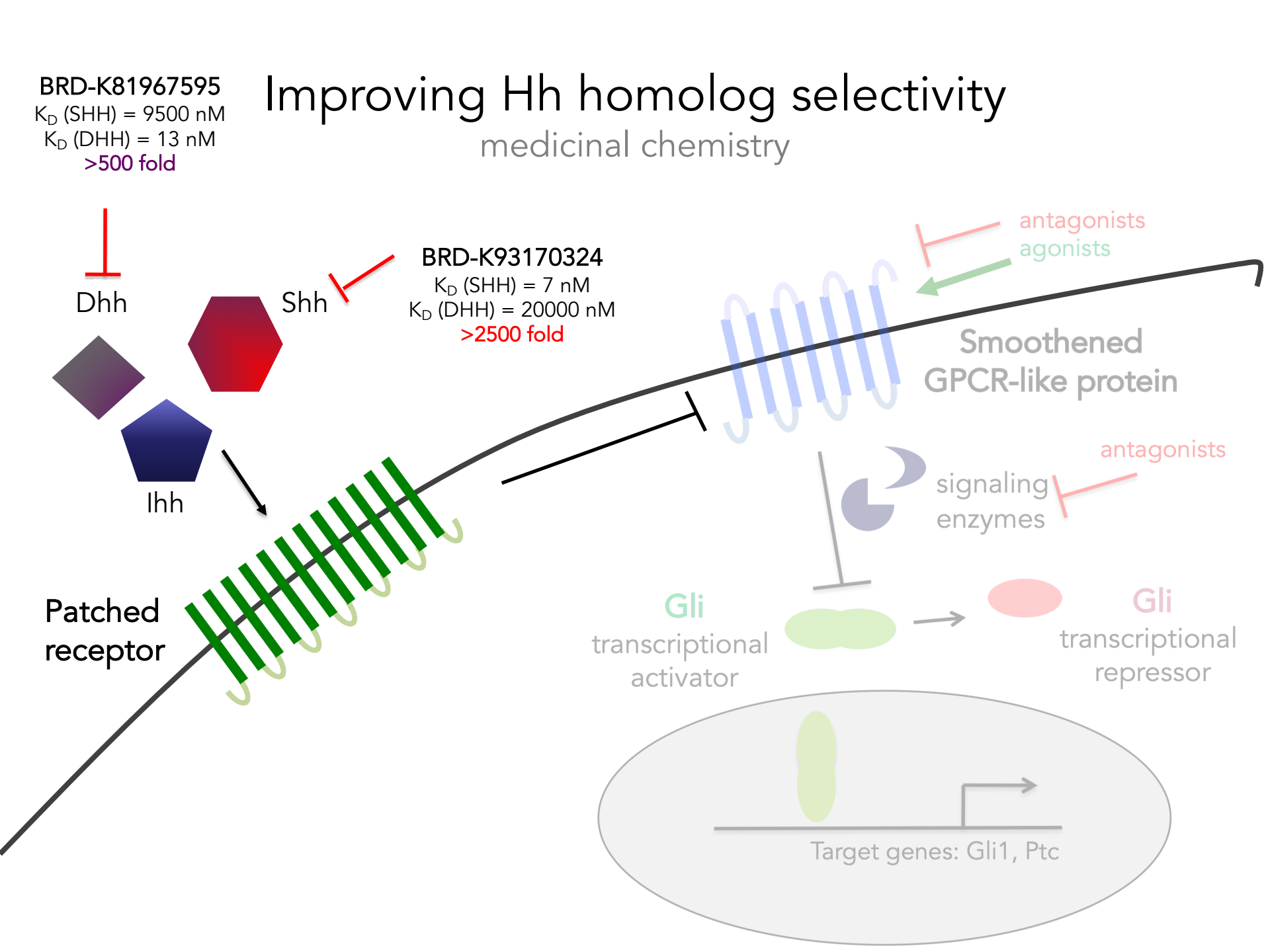




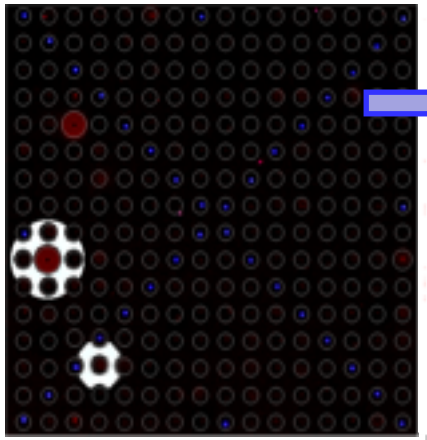
robotnikinin treatment shows no signs of inflammation or failed skin differentiation

Improving Hh homolog selectivity

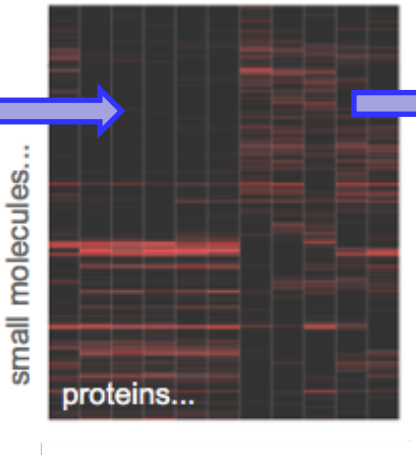
medicinal chemistry



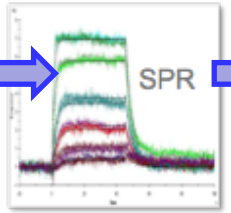
Path for probe discovery, validation, and development

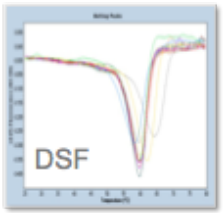


SMM

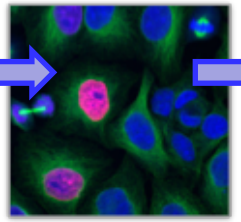


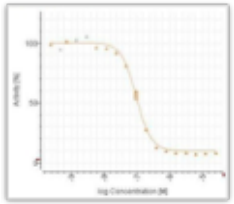
specificity analysis



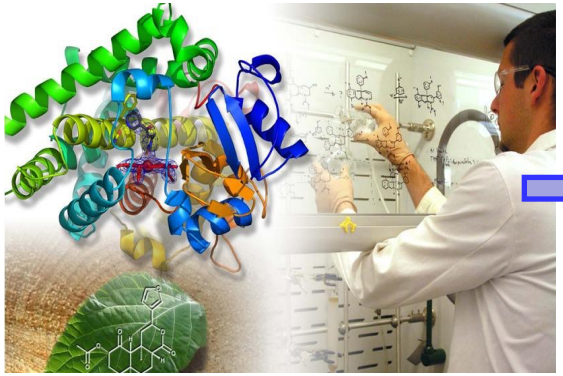


secondary binding





cellular or biochemical assays



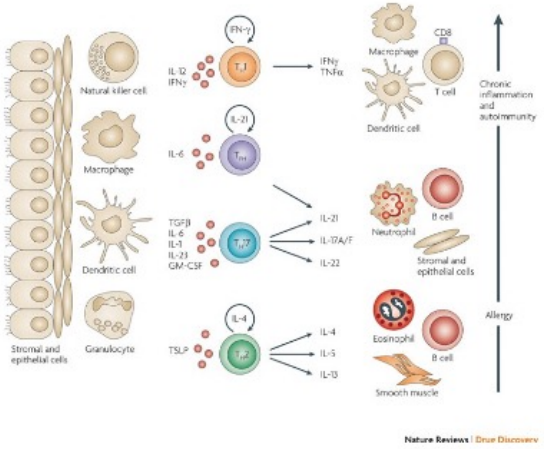
optimize molecules using chemistry

additional cell biology animal models

Into the eye of the cytokine storm

New Direction - IL-4 and profiling pro-and anti-inflammatory cytokines

Arturo Vegas, Boston University



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Letters

A Small-Molecule Inhibitor to the Cytokine Interleukin-4

Sean P. Quinnell, Becky S. Leifer, Stephen T. Nestor, Kelly Tan, Daniel F. Sheehy, Luke Ceo, Shelby K. Doyle, Angela N. Koehler, and Arturo J. Vegas*

Cite This: https://dx.doi.org/10.1021/acschembio.0c00615

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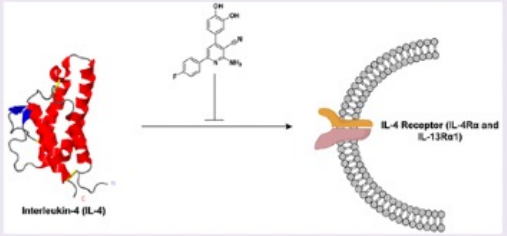
ABSTRACT: Interleukin-4 (IL-4) is a multifunctional cytokine and an important regulator of inflammation. When deregulated, IL-4 activity is associated with asthma, allergic inflammation, and multiple types of cancer. While antibody-based inhibitors targeting the soluble cytokine have been evaluated clinically, they failed to achieve their end points in trials. Small-molecule inhibitors are an attractive alternative, but identifying effective chemotypes that inhibit the protein−protein interactions between cytokines and their receptors remains an active area of research. As a result, no small-molecule inhibitors to the soluble IL-4 cytokine have yet been reported. Here, we describe the first IL-4 small-molecule inhibitor identified and characterized through a combination of binding-based approaches and cell-based activity assays. The compound features a nicotinonitrile scaffold with micromolar affinity and potency for the cytokine and disrupts type II IL-4 signaling in cells. Small-molecule inhibitors of these important cell-signaling proteins have implications for numerous immune-related disorders and inform future drug discovery and design efforts for these challenging protein targets.



cytokines have compact globular folds
typically modulated with antibodies and considered ‘undruggable’ with small molecules