Eph receptors and ephrins in angiogenesis: Regulation by hypoxia

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SCIENCE AND THE MEDIA

The Power of the Front Page Of *The New York Times*

"Ultimate Weapon against Cancer:
Go for Blood Vessels"

U.S. cancer clinics were swamped with phone calls last week from [illegible] they [illegible] media. [illegible] called a[illegible]statin and endostatin that block the development of blood vessels [illegible] shown promise in tr[illegible] Neither has been te[illegible] ever, and they are n[illegible] cal trials for at least a year. But last week, reports that they might "cure" cancer within 2 years raced through the media.

The trigger for all this excitement was a feature story on the two compounds that appeared high on the front page of *The New York Times* on Sunday, 3 May. Although the story contained several caveats—noting in particular that findings in mice often don't hold up in humans—the message was given an optimistic spin by enthusiastic quotes from Nobel Prize–winner James Watson and National Cancer Institute (NCI) director Richard Klausner. That evening, TV news broadcasts featured the story, and the next day hundreds of newspapers carried hopeful news about angiostatin and endostatin. Within days, however, the media frenzy had shifted. Articles debunking the notion of an imminent cancer cure appeared in the *Los Angeles Times*, *The Boston Globe*, and *The Washington Post*. Watson and Klausner backed away from their quotes. Even Kolata and [illegible] Watson [illegible] Nobel Prize with Francis Crick for identifying the structure of DNA. By her own account, Kolata turned [illegible]tson during dinner like: "What's new [illegible] search?"

[illegible] guarded comments have gotten him in hot water before—responded by talking about the buzz among cancer researchers over experiments by Judah Folkman. For 30 years, Folkman and his colleagues at Harvard University's Children's dinnertime comments were going to be quoted and was "horrified" when they were.

Klausner was quoted in the story as saying angiostatin and endostatin are "the single most exciting thing on the horizon" for the treatment of cancer, and that they were the top priority of NCI. These heady phrases were offset by a careful headline, however: "A Cautious Awe Greets Drugs That Eradicate Tumors in Mice." The story also included warnings that the promised cure might not materialize, such as a sentence in the second paragraph stating that "the history of cancer treatments is full of high expectations followed by dashed hopes when drugs with remarkable effects in animals are tested in people."

But the caveats didn't blunt the impact. When TV broadcasts carried the news, says Eric Rosenthal, spokesperson for the Fox Chase Cancer Center in Philadelphia, they often used "less sophisticated teasers" to promote the story, along the lines of: "New cancer cure—more at 11." Even though some of the stories made it clear that the new results were from mice, the effect was explosive. The Memorial Sloan-Kettering Cancer Center in New York City, for example, was "flooded with calls," says its president, Paul Marks. "We even had patients calling us to say they didn't want to start their chemotherapy—that they wanted to wait for the new drugs."

The frenzy began to cool almost immediately, however, as other journalists began investigating. According to a staffer at Memorial Sloan-Kettering, another *Times* reporter who h[illegible] [illegible]ng on a [illegible] [illegible]elated [illegible]ery a[illegible] Kettering, Ian Fisher, inquired on behalf of a col-

A Cautious Awe Greets Drugs That Eradicate Tumors in Mice

By GINA KOLATA

Within a year, if all goes well, the first cancer patient will be injected with two new drugs that

HOPE IN THE LAB
A special report.

In Excitement Over Cancer Drugs, A Caution Over Premature Hopes

By IAN FISHER

Dr. Larry Norton, a prominent oncologist in New York City, received a telephone call at his hom[illegible]

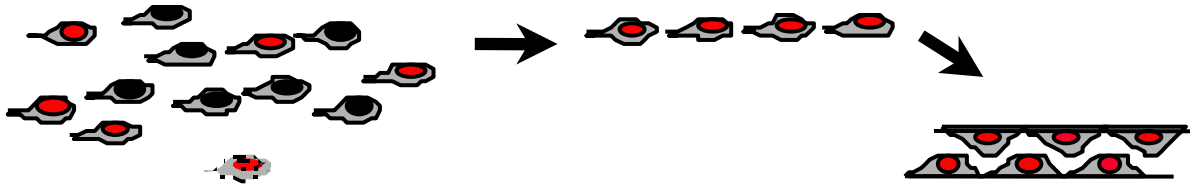
ble edged. On one hand, he said, it is good that cance[illegible]ath[illegible] and the public learn ab[illegible]

Two views. The *Times*' first story [illegible], focused on the promise of antiangiogenesis factors, its second emphasized the problems.

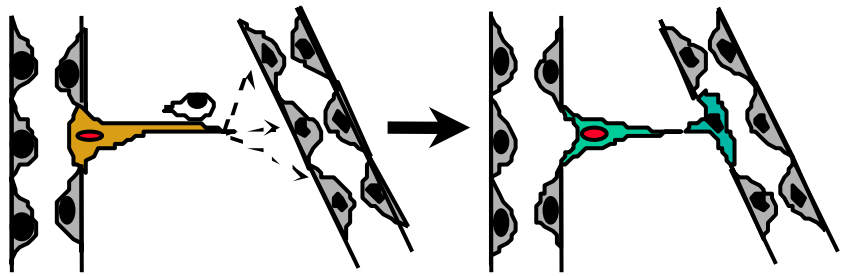
"The Magic Bullet"

„Endotheliocentric" view of vascular assembly

Vasculogenesis



Angiogenesis



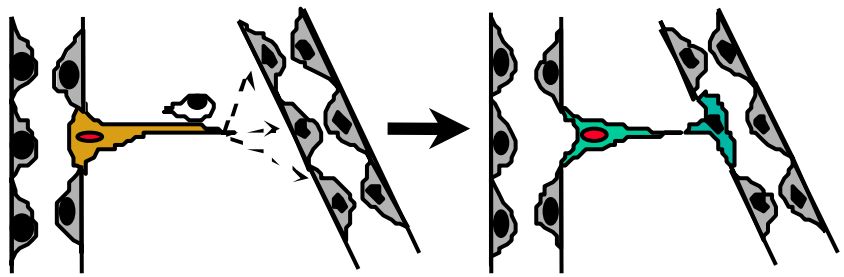
- Developmental Angiogenesis
- Angiogenesis in the Adult: Cancer / Wound healing

„Endotheliocentric" view of vascular assembly

Proliferation

VEGF receptor / VEGF
FGF receptor / FGF
PDGF receptor / PDGF

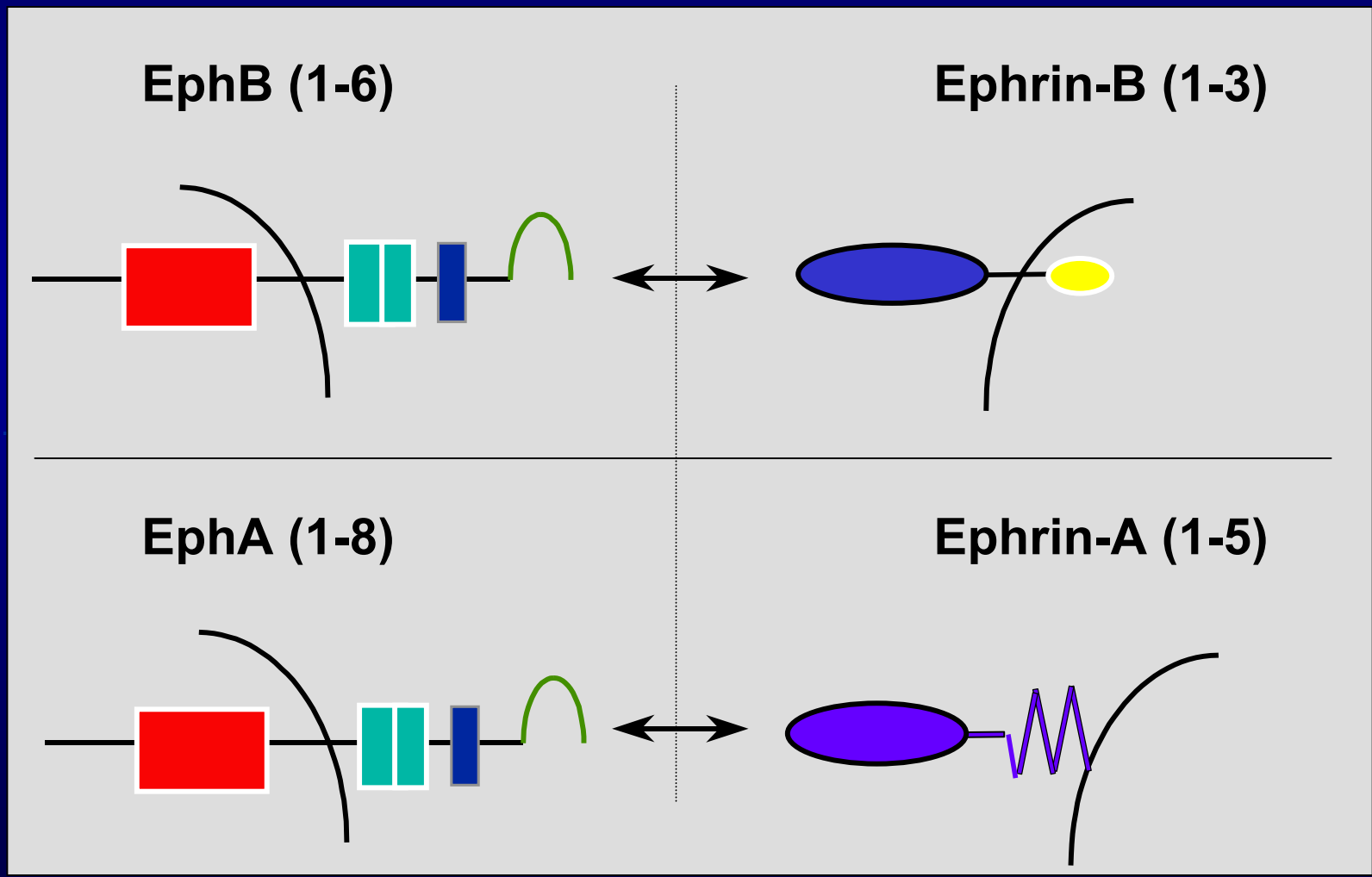
Angiogenesis



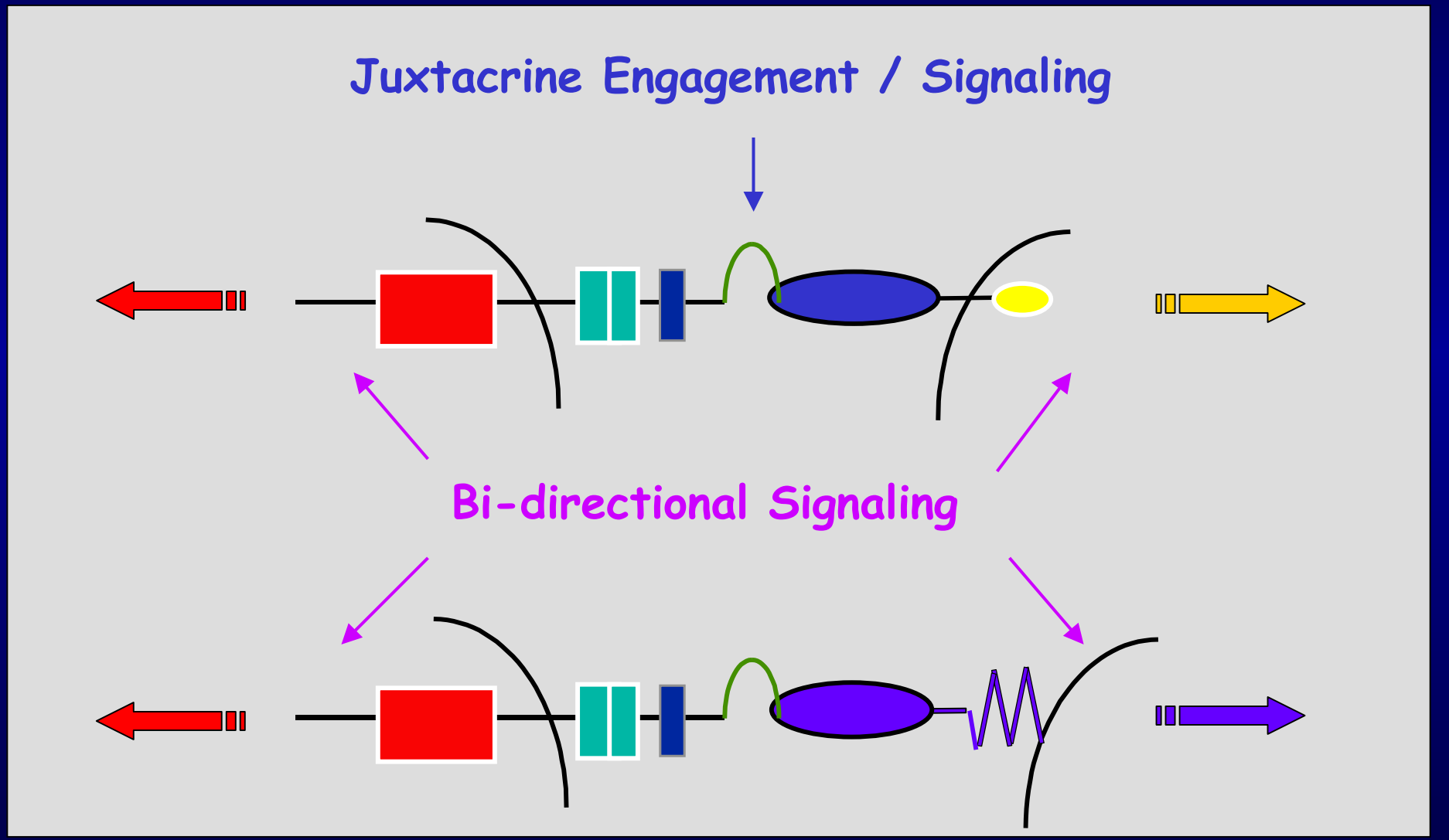
Targeting

Tie-2 receptor / angiopoietins
Eph receptors / ephrins

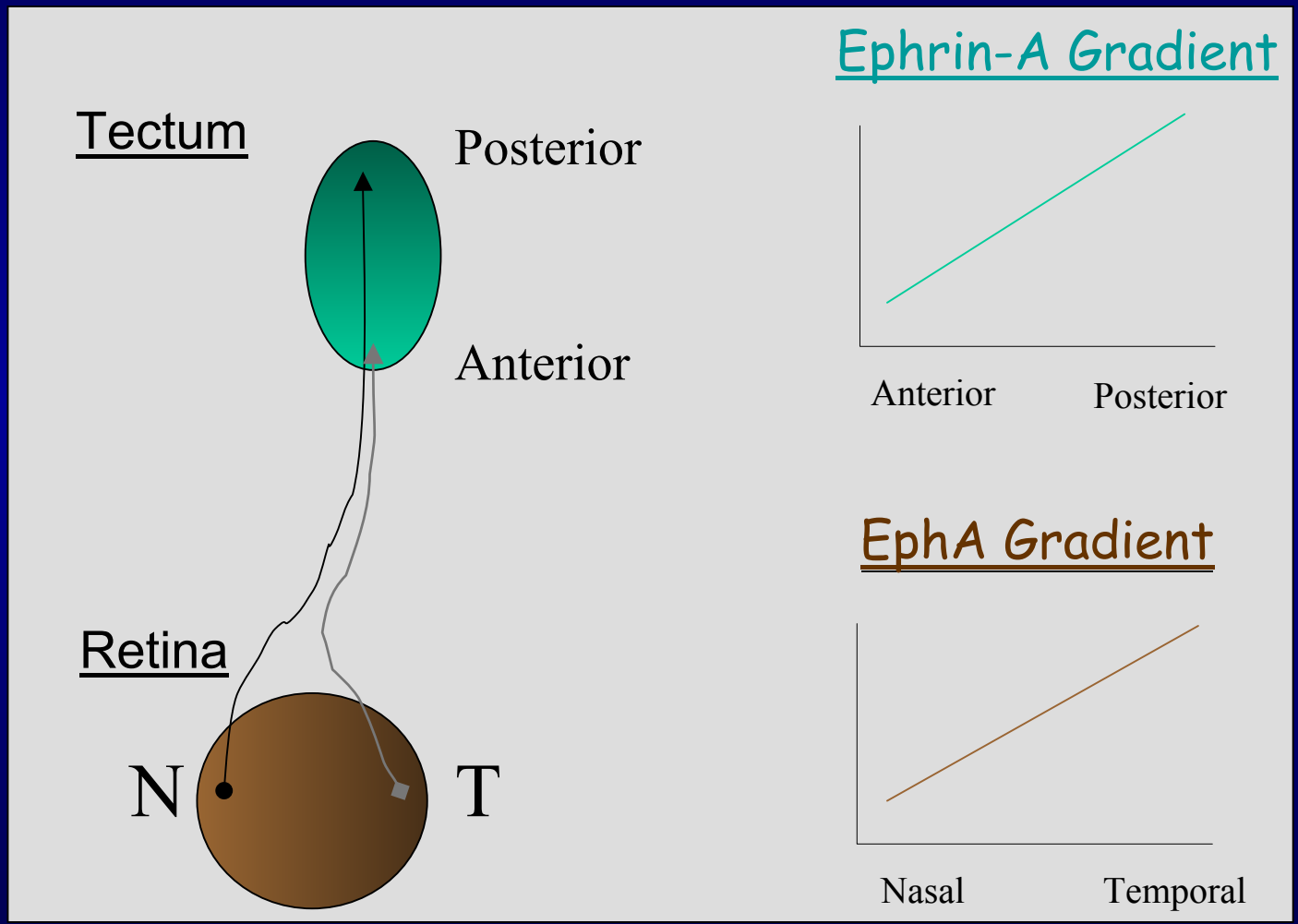
Erythropoeitin Producing hepatocellular Carcinoma



Eph receptors & Ephrin ligands

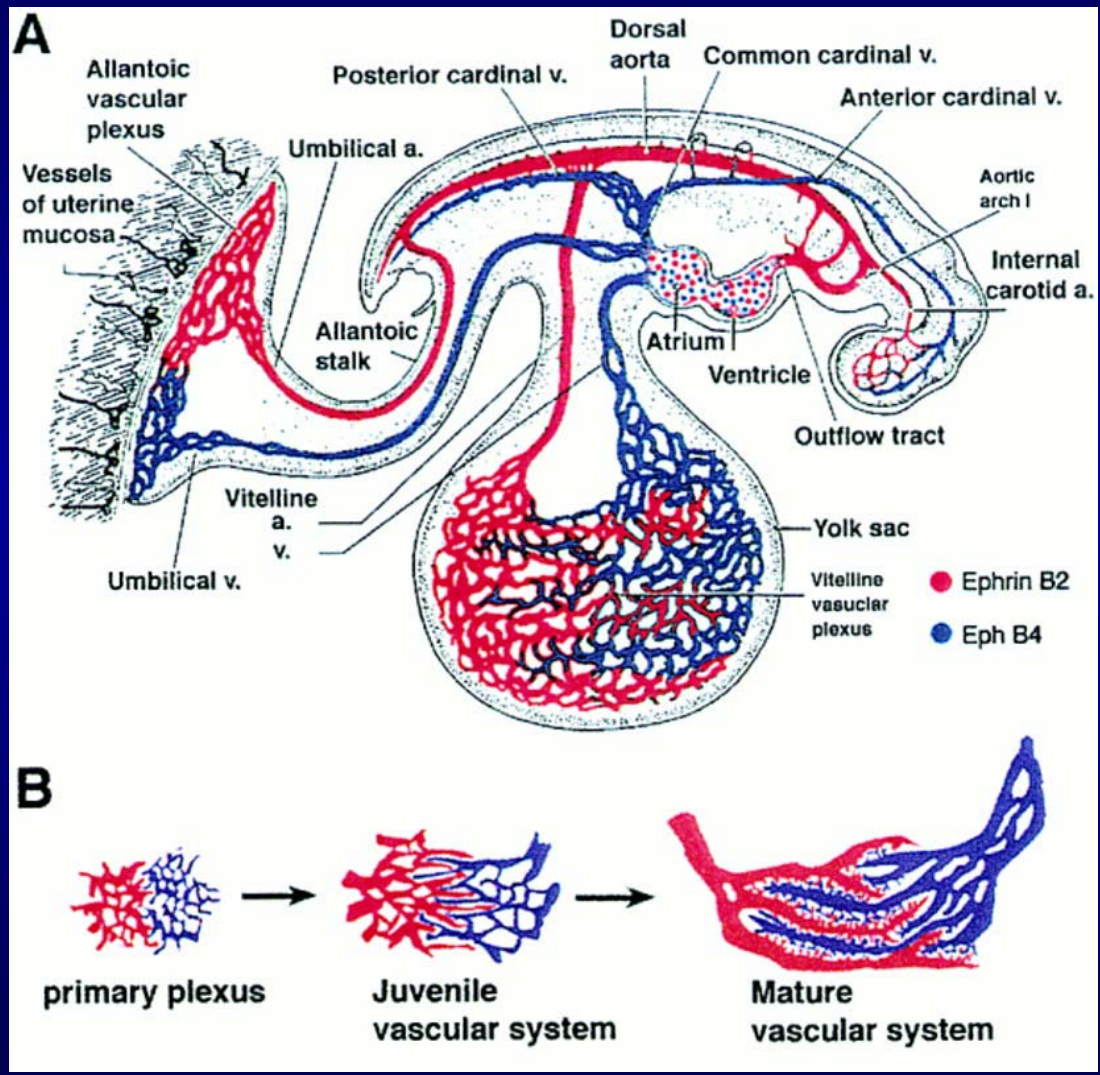


Eph/Ephrin Gradients Determine Retinal Axon Guidance



Cheng et al, *Cell*, 1995

EphB4 / ephrinB2 Interaction at boundary Regions determine arteriovenous Anastomosis



Wang et al, *Cell*, 1998

Vascular Endothelial Targeting: Critical Role of Eph / ephrin Interactions

In contrast to most other vascular growth factors, Eph receptors have no proliferative functions. However they play an essential role in guiding cell- cell and cell-matrix interaction.
→ mature, vascular networks

In the embryo, Eph receptors play a critical role in axonal guidance and vascular patterning.
In the adult organism however, their functions and regulation still remain to be defined.

Tissue Hypoxia is an Important Determinant of Eph / ephrin Expression

Rationale

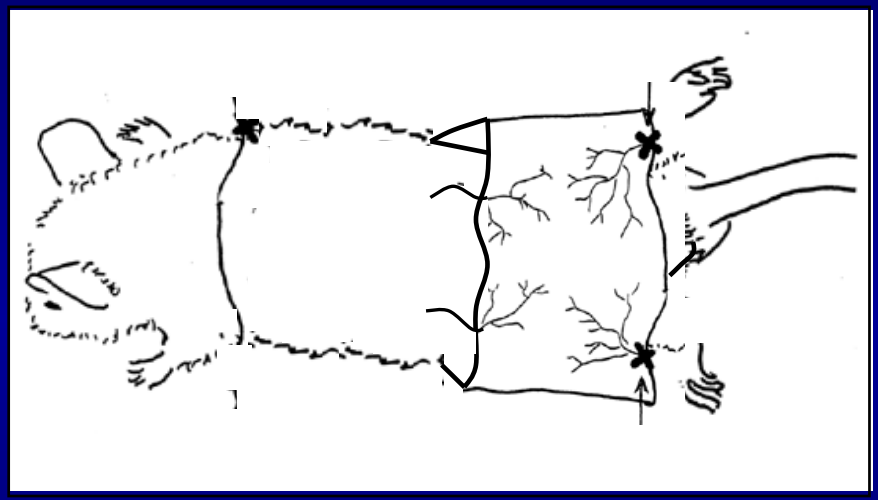
- Eph and ephrins are upregulated in a variety of cancers
- Tissue hypoxia plays an important role in tumor angiogenesis

→ Which *in vivo* Model for Tissue Hypoxia?

- Reflect true tissue hypoxia, NOT ischemia or ischemia / reperfusion (confounders...)
- Allow quantitative & „real-time“ assessment of hypoxia
- Be a murine model (future studies in transgenic mice)

Tissue Hypoxia is an Important Determinant of Eph / ephrin Expression

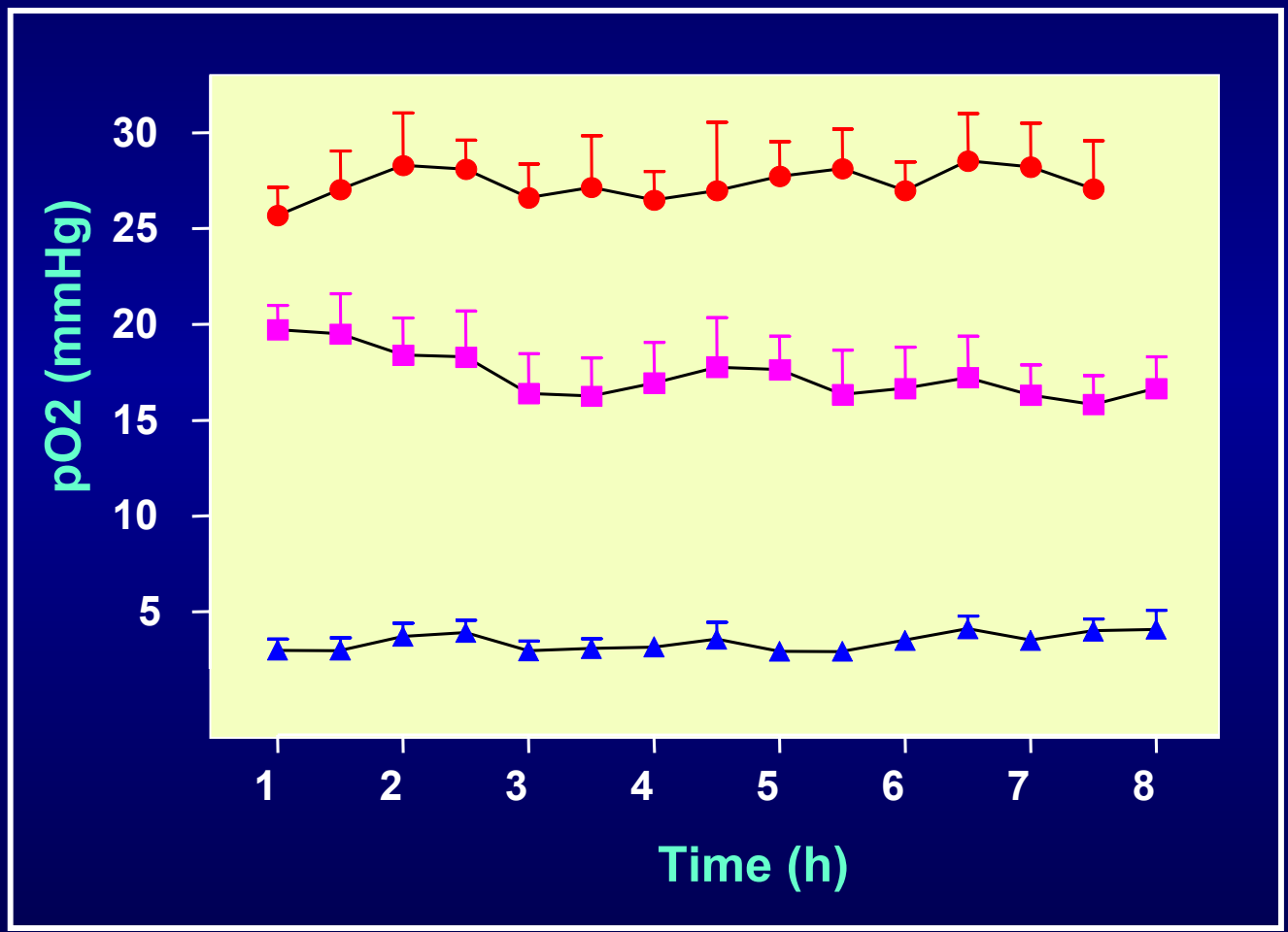
→ A new Mouse Skin Flap Model



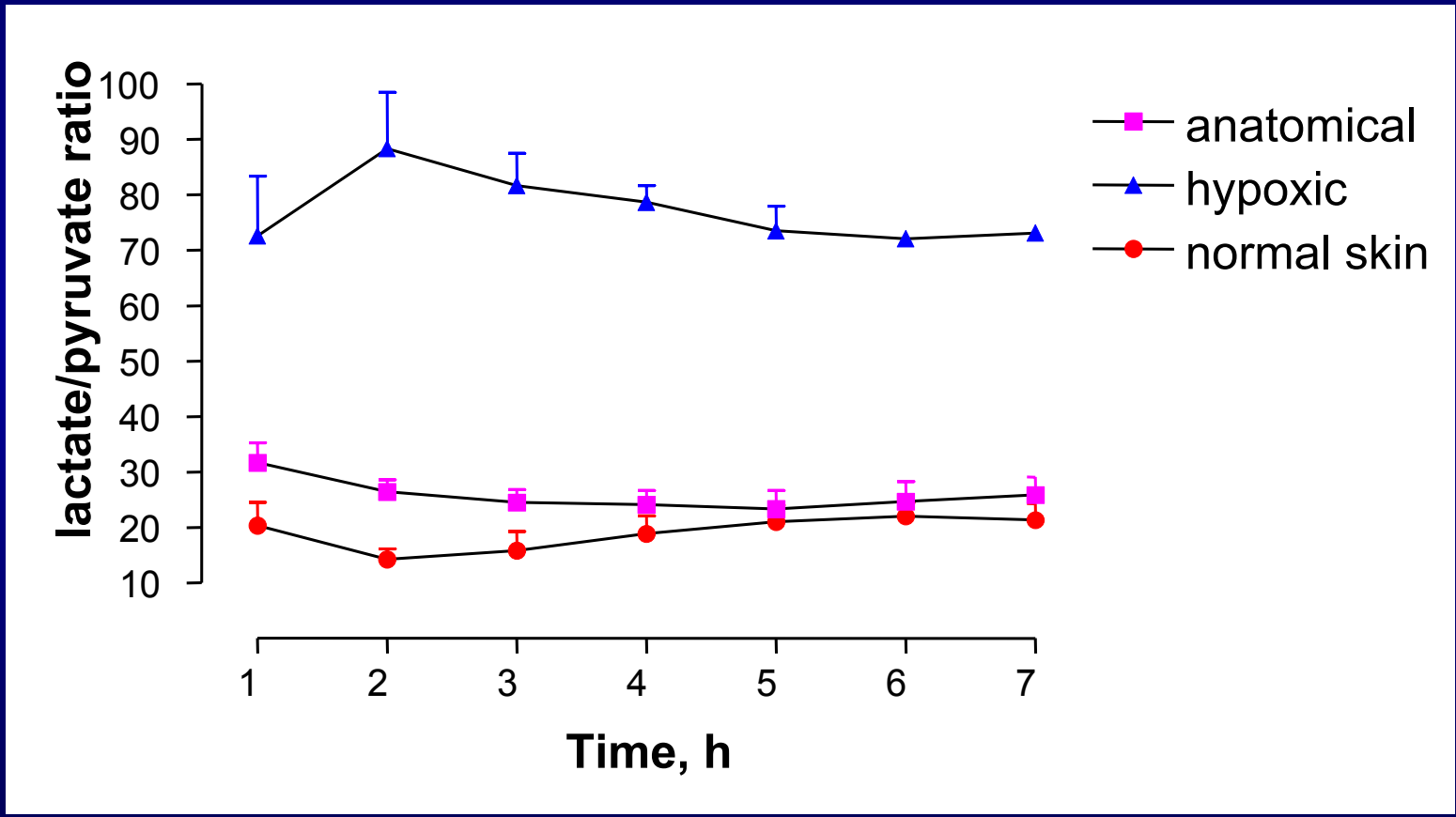
Flap is perfused by iliac arteries

Oxygen & Microdialysis Probes are inserted →

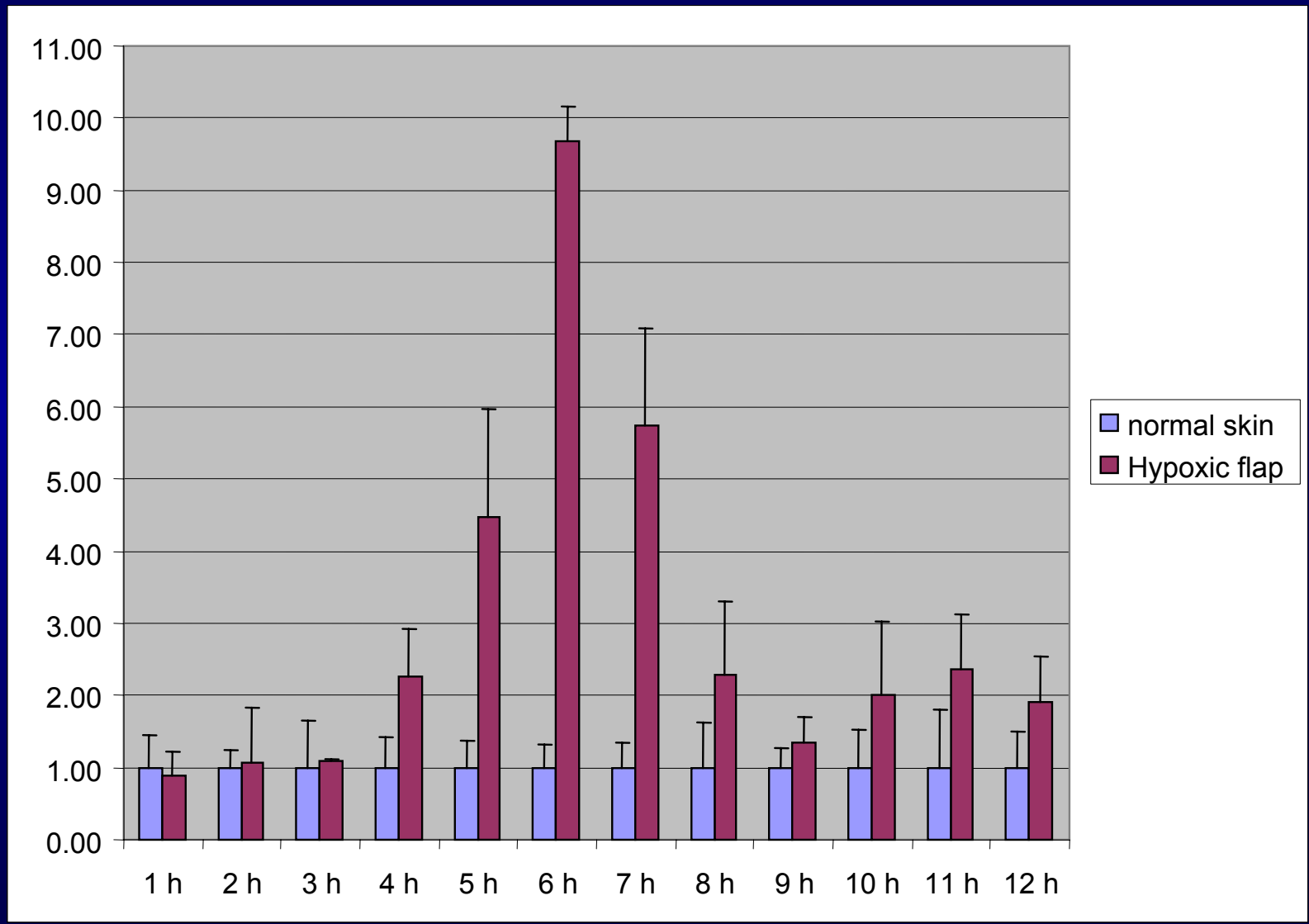
Continuous monitoring of Oxygen tension



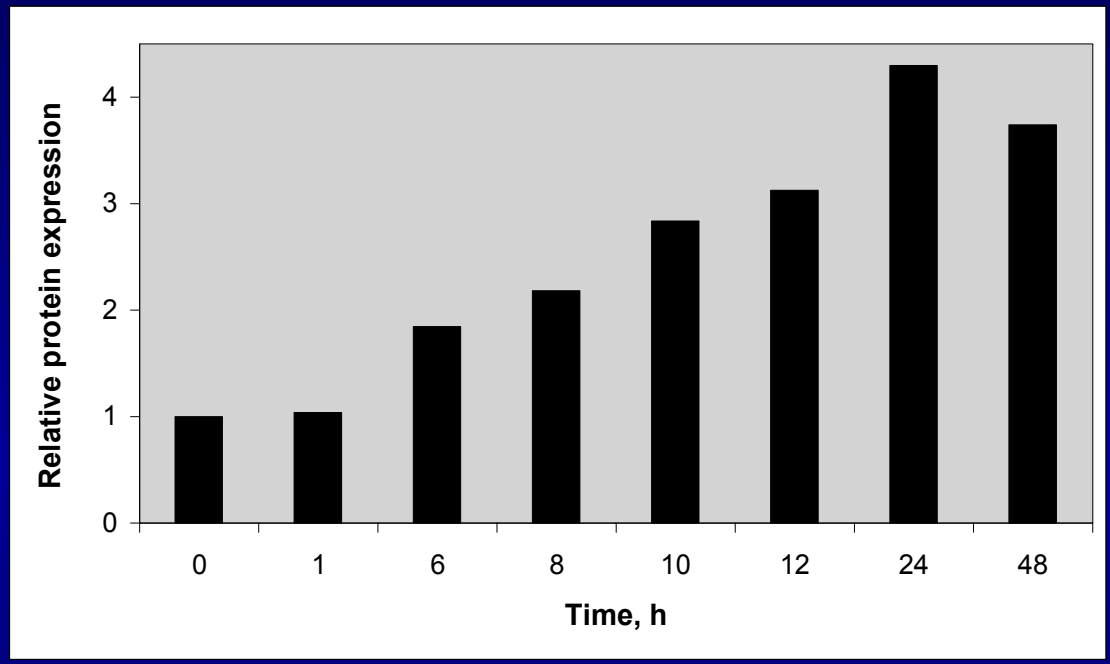
Continuous monitoring of Metabolism: Lactate - Pyruvate ratio



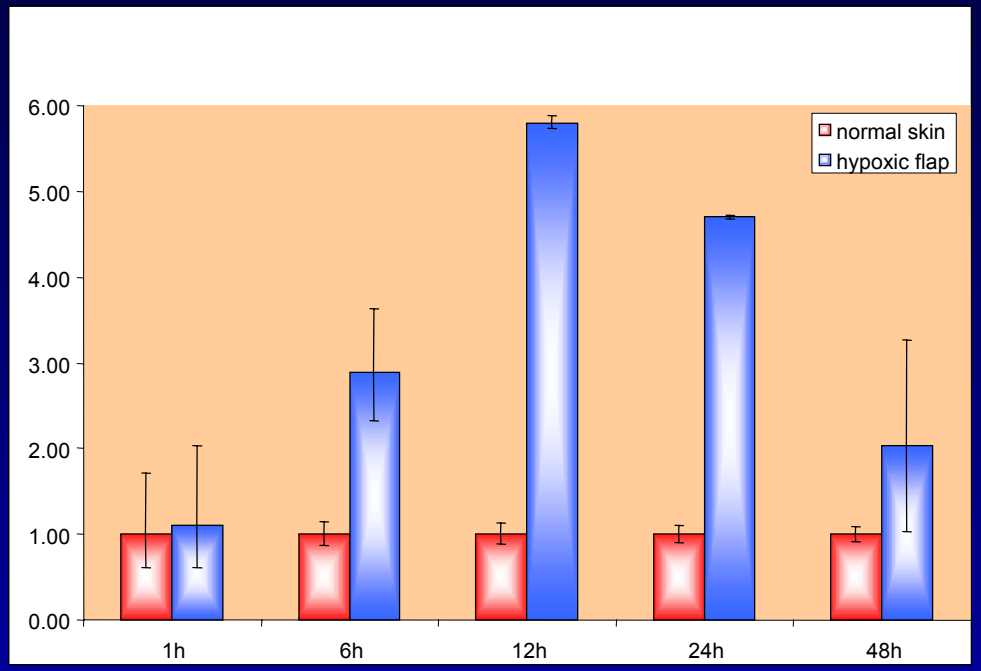
Time course of HIF-1α m-RNA expression

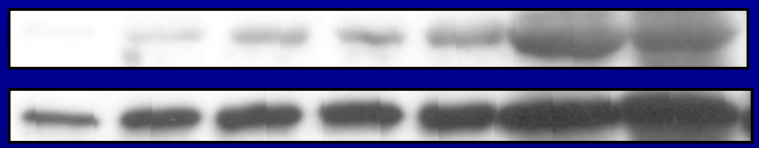


Time course of VEGF protein expression



EphB4 receptor mRNA & Protein expression increase during hypoxia

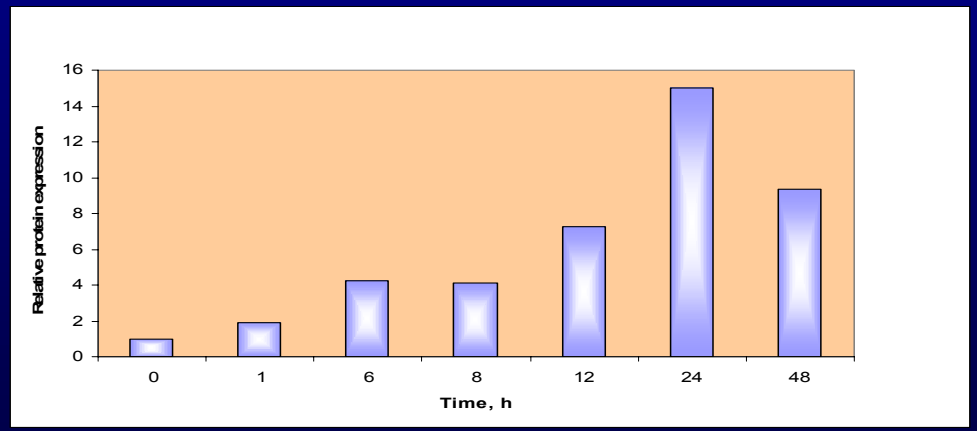


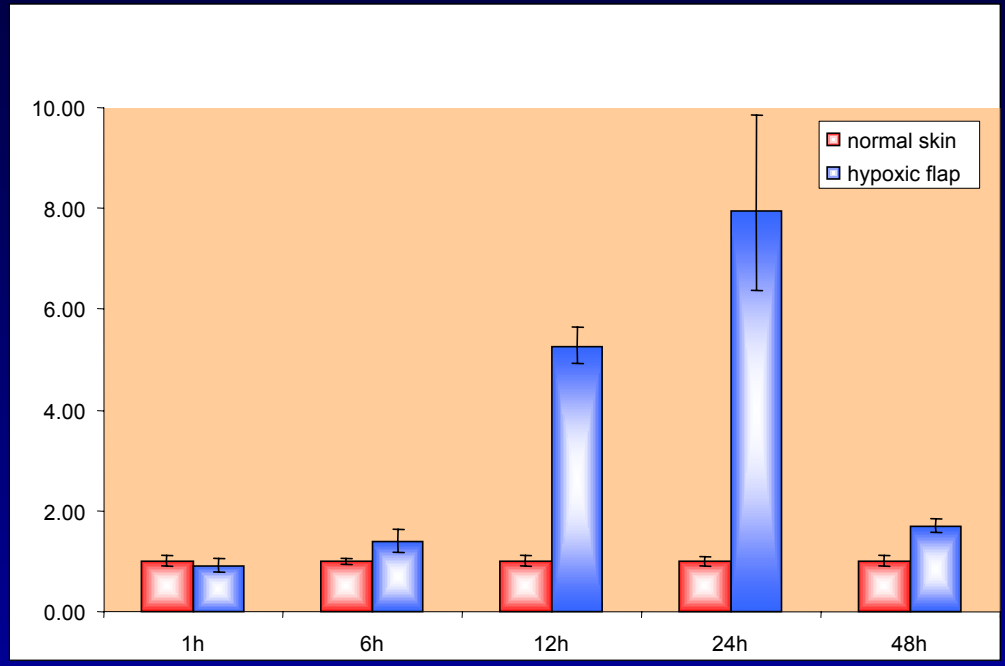


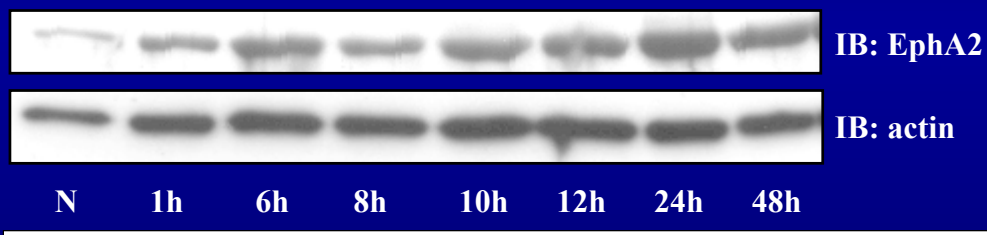
IB: EphB4

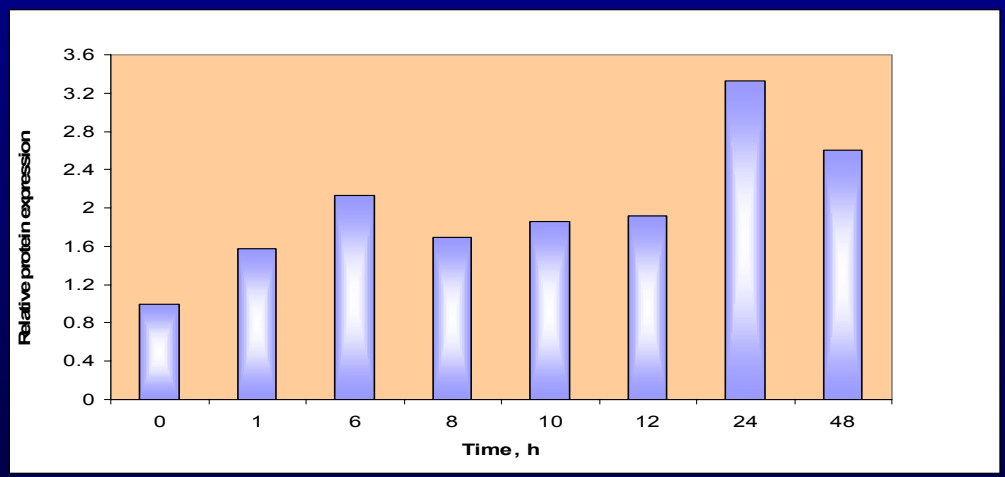
IB: actin

N 1h 6h 8h 12h 24h 48h







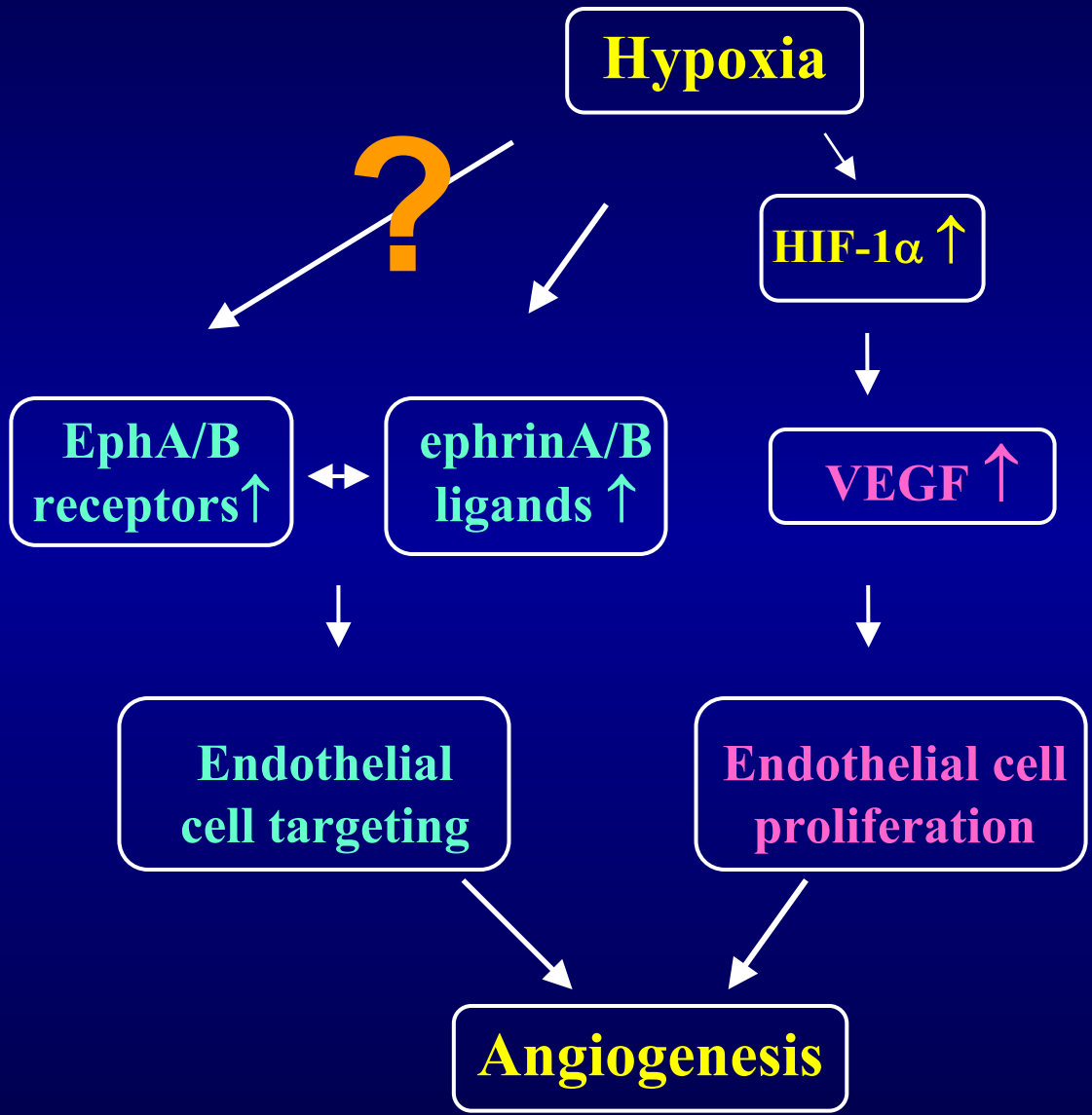


EphA2 receptor mRNA & Protein expression increase during hypoxia

Tissue Hypoxia is an Important Determinant of Eph / ephrin Expression
Conclusions - I

1) The mouse dorsal skin flap model allows precise quantitative assessment of segmental skin hypoxia. Results are highly reproducible

2) EphB4 / ephrinB2, and EphA2 / ephrinA1 are upregulated in response to local tissue hypoxia

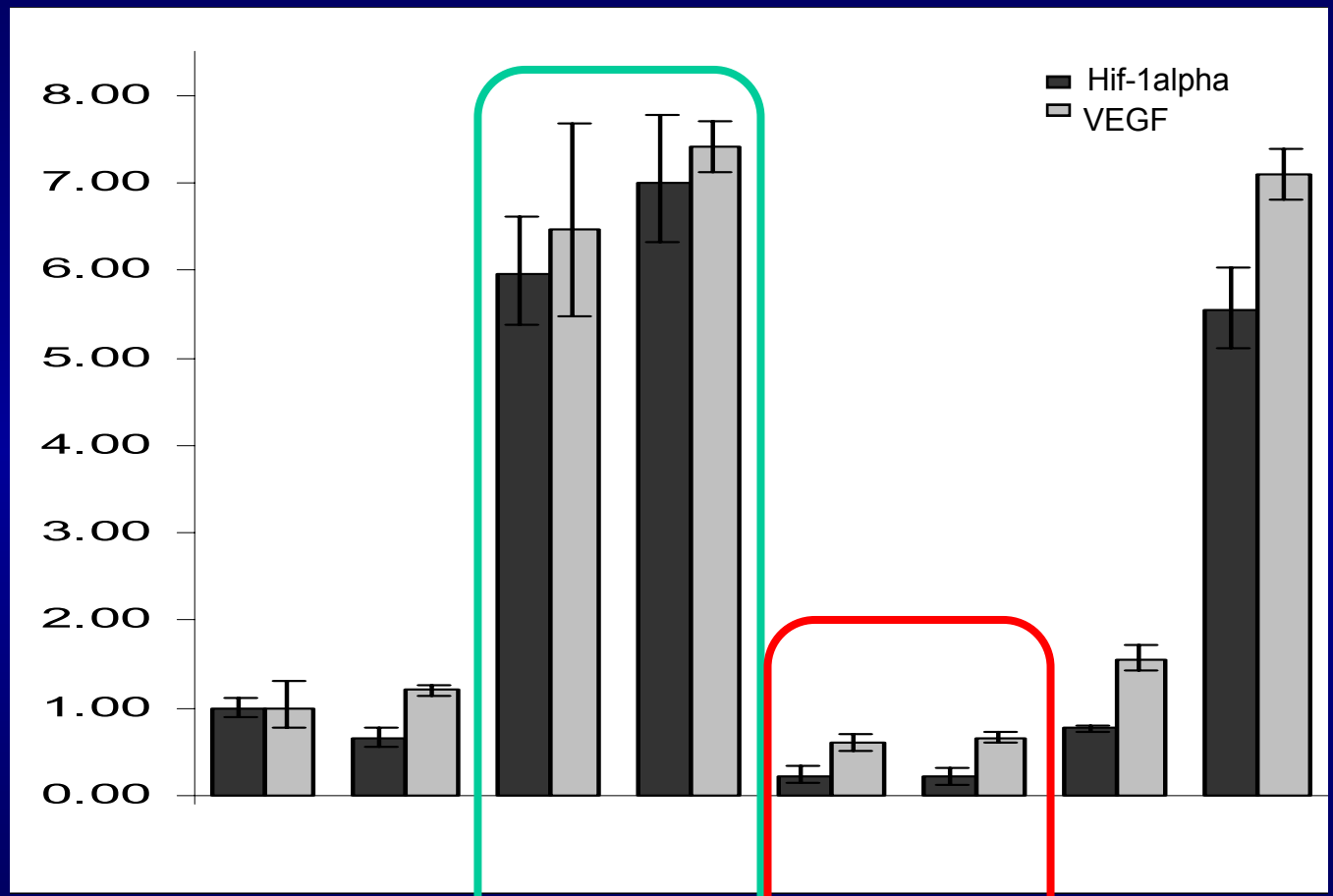

Hypoxia
?
HIF-1α ↑
EphA/B receptors↑
ephrinA/B ligands ↑
VEGF ↑
Endothelial cell targeting
Endothelial cell proliferation
Angiogenesis

Does Hypoxia induce Eph / ephrin Expression through HIF-1α pathway?

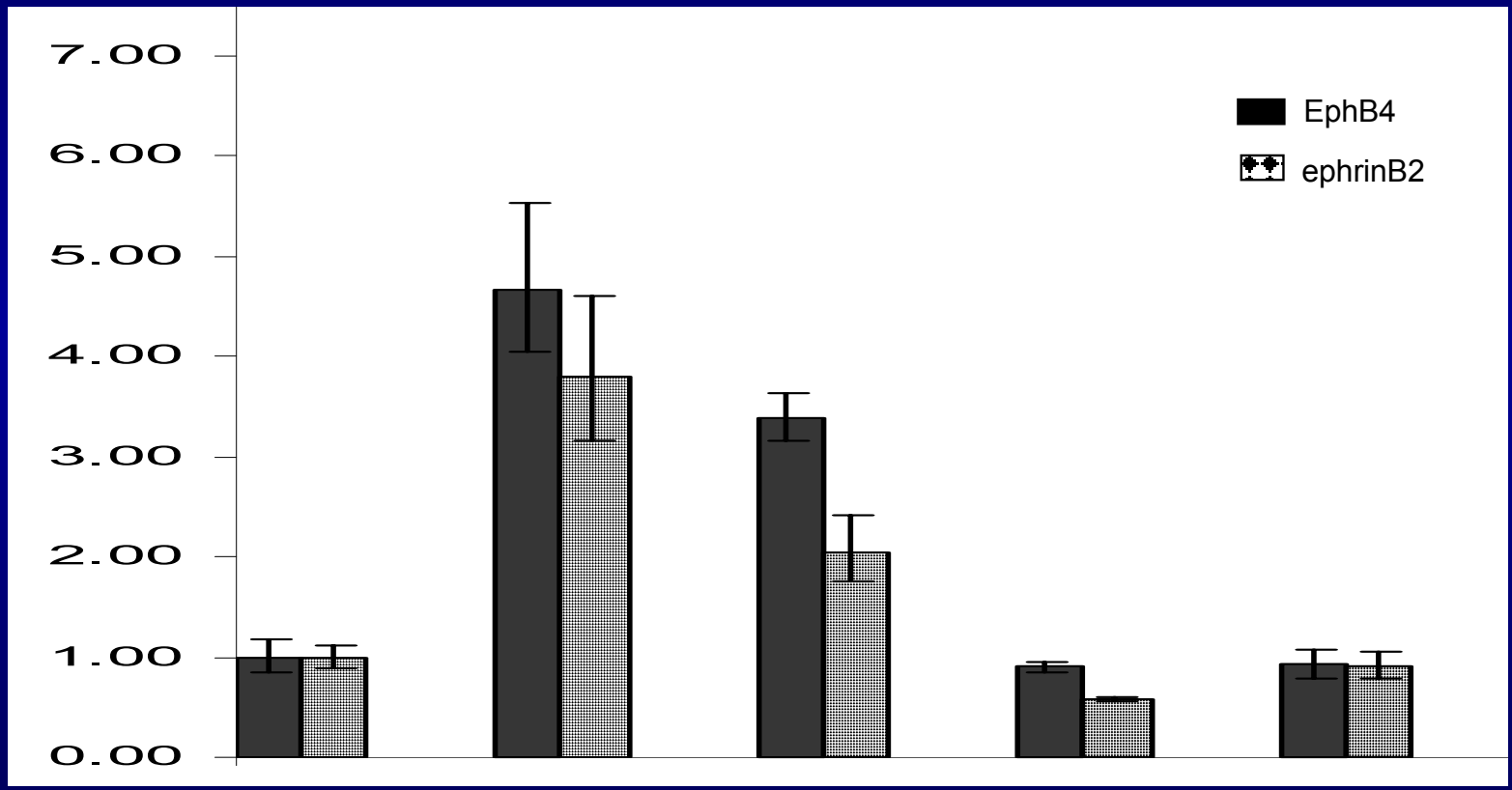
→ *In vitro* induction of chemical hypoxia

- Cell culture system: Hep3B and PC3 cells
- Induction of chemical hypoxia with Cobalt chloride
- RNA interference (posttranscriptional gene silencing) siRNA against HIF-1α vs. scrambled siRNA

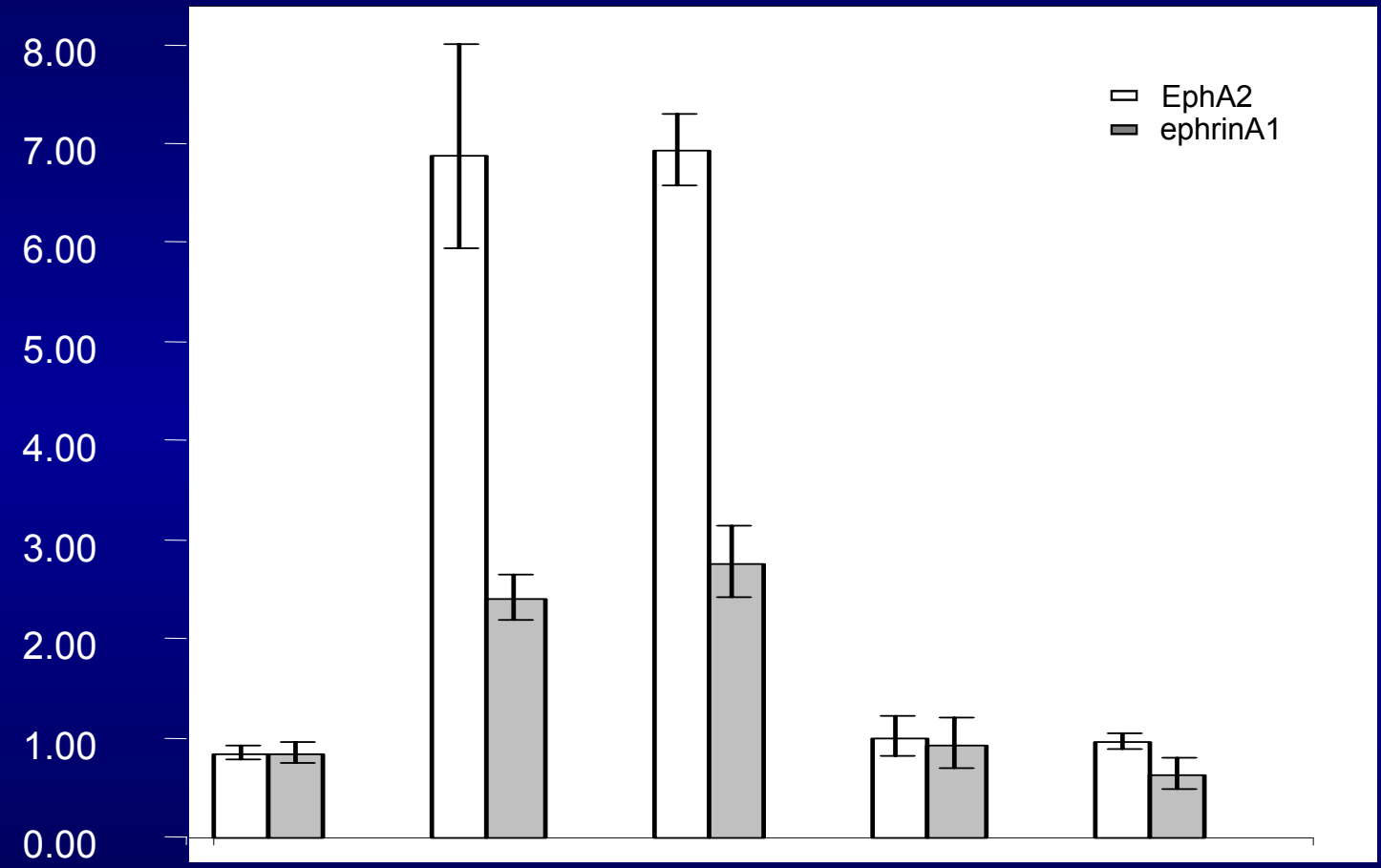
Hypoxia-induced expression of HIF-1α and VEGF is inhibited by siRNAs against HIF-1α

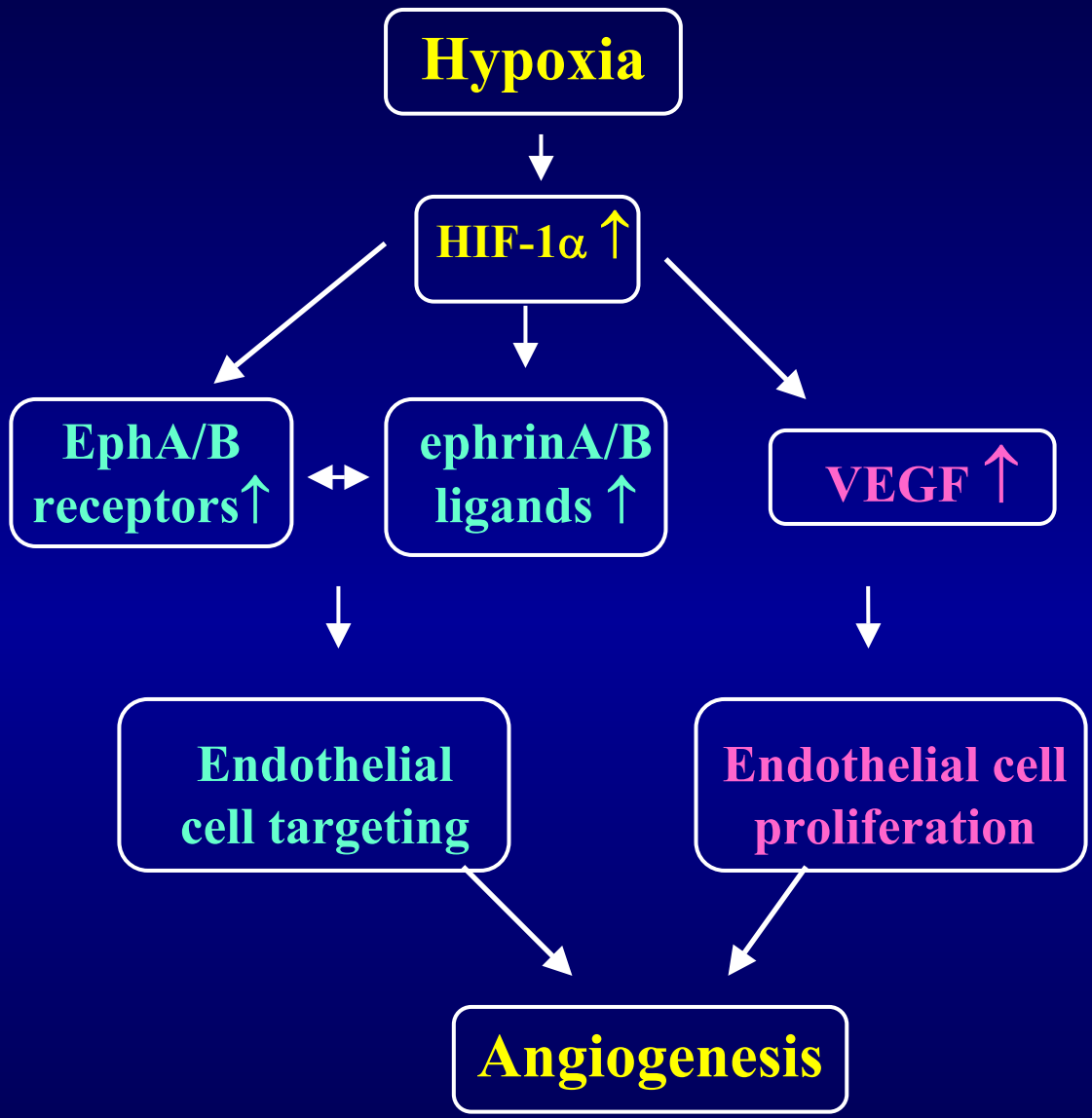


siRNA against HIF-1α inhibit hypoxia-induced expression of EphB4 & ephrinB2 in Hep3B cells



siRNA against HIF-1α inhibit hypoxia-induced expression of EphA2 & ephrinA1 in PC3 cells





Vihanto et al, *FASEB J* 2005