Gli APTAMERI

The term “Aptamer” was coined by Andy Ellington. It stems from the Latin terms “aptus,” meaning to fit, and Greek “meros,” meaning part.

Name of the aptamer	Primary target of the aptamer	Status
Macugen	VEGF	Approved [22]
AS1411	Nucleolin	Phase II [25, 26]
REG1	Factor Ixa	Phase II [29, 30]
EYE001	VEGFR	Phase II/III [47, 49]
LY2181308	Survivin mRNA	Phase III [50, 51]
E_2F decoy oligonucleotides	Mesangial cells	Phase III [52, 53]
ARC1779	Vwf	Phase II [31]
NU172	Thrombin	Phase II [32]
E10030	PDGF	Phase II [23]
ARC1905	C5	Phase I [24]
NOX-E36	MCP-1	Phase I [27, 33]
NOX-A12	SDF-1	Phase I [27, 28]
NOX-H94	Hepcidin	Phase I [21]
BAX499/ARC19499	TFPI	Phase I [34, 35]
DNA aptamers	Thrombin	Research [11]

Aptameri e malattie della retina

La maculopatia senile umida è causata dalla crescita di vasi sanguigni anomali, che danneggiano l'area dell'occhio responsabile della visione centrale, che è essenziale per la maggior parte delle attività visive

VEGF and Macula Degeneration

- Both clinical and preclinical findings have implicated vascular endothelial growth factor (VEGF) in the pathophysiology macular edema and degeneration.

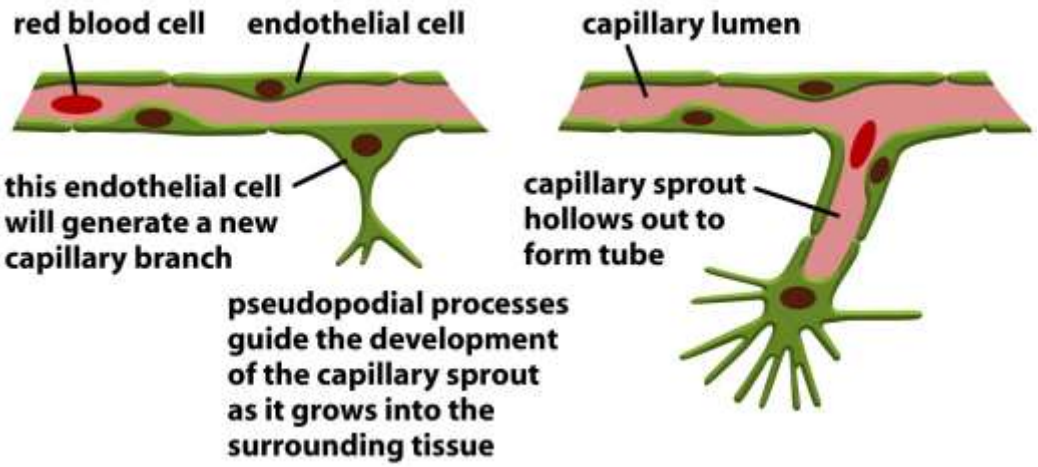
- *VEGF is both a potent enhancer of vascular permeability and a key inducer of angiogenesis.

VEGF and Macula Degeneration

- *VEGF levels are elevated in the eyes of patients.
- Injection of VEGF (**the VEGF165 isoform** in particular) into healthy eyes of animals can induce associated ocular pathologies

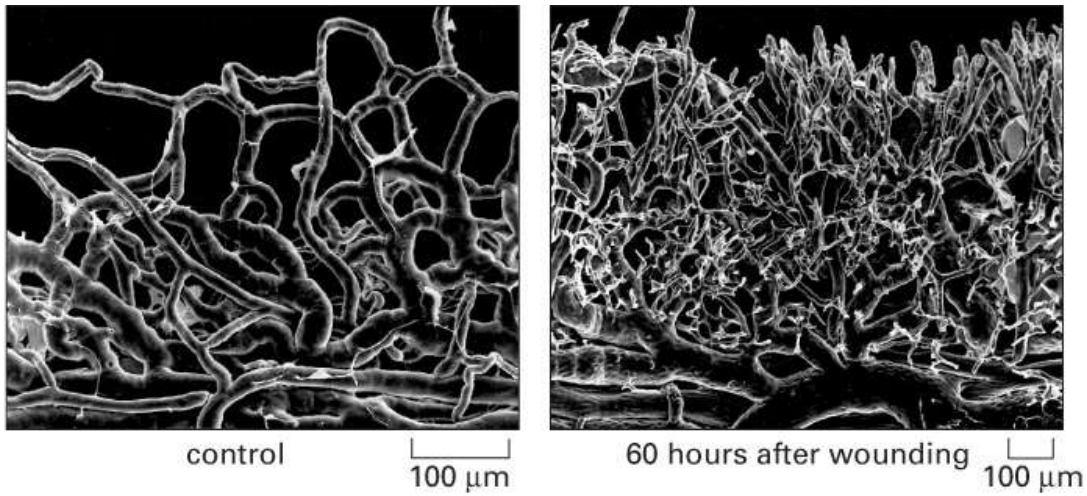
Angiogenesis:

Sprouting of cells from mature endothelial cells of the vessel wall



Secretion of proteases,
Resolution of Basal lamina,
Migration towards
Chemotactic gradient,
Proliferation,
Tube formation

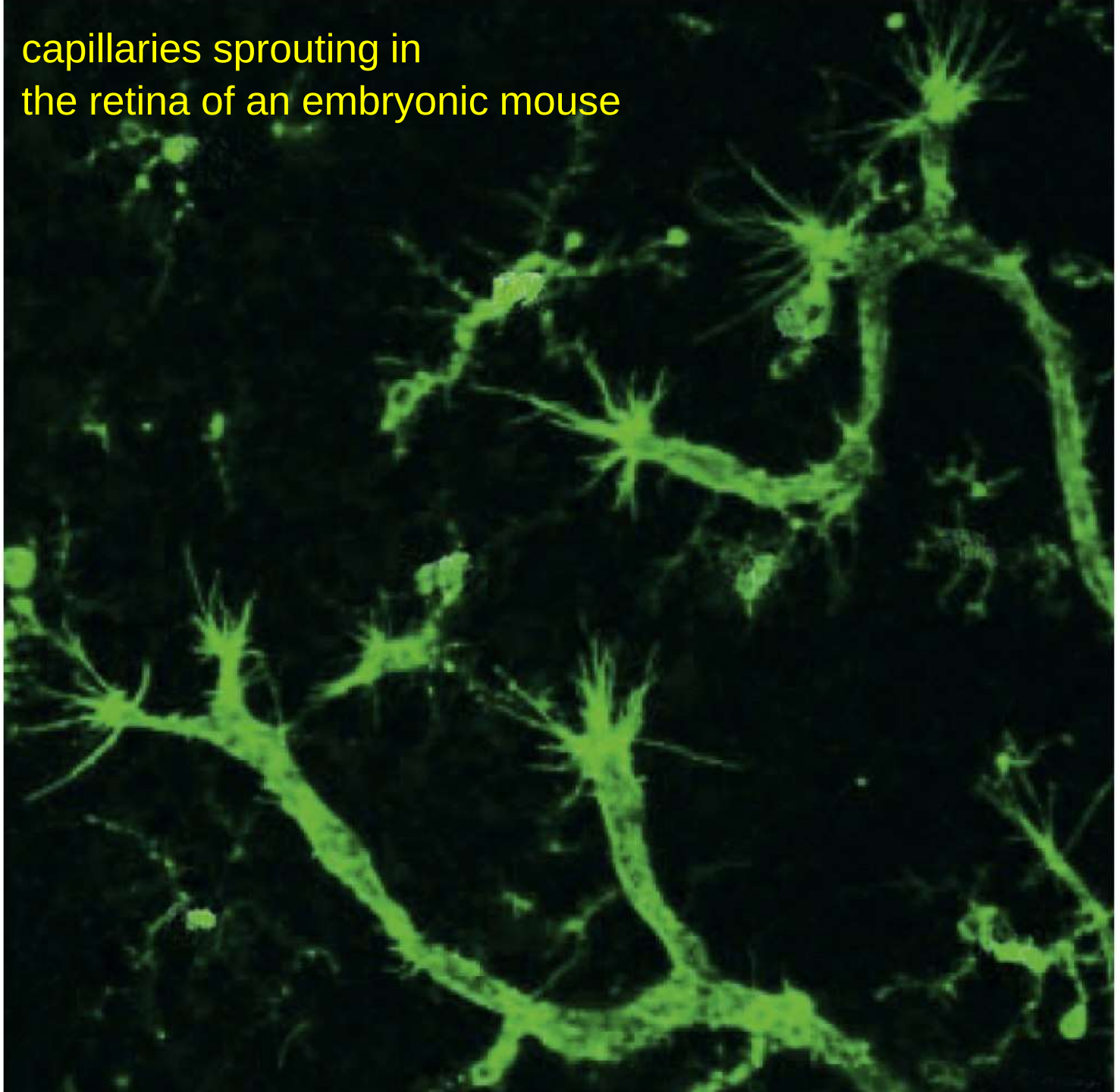
VEGF is factor largely specific for endothelial cells



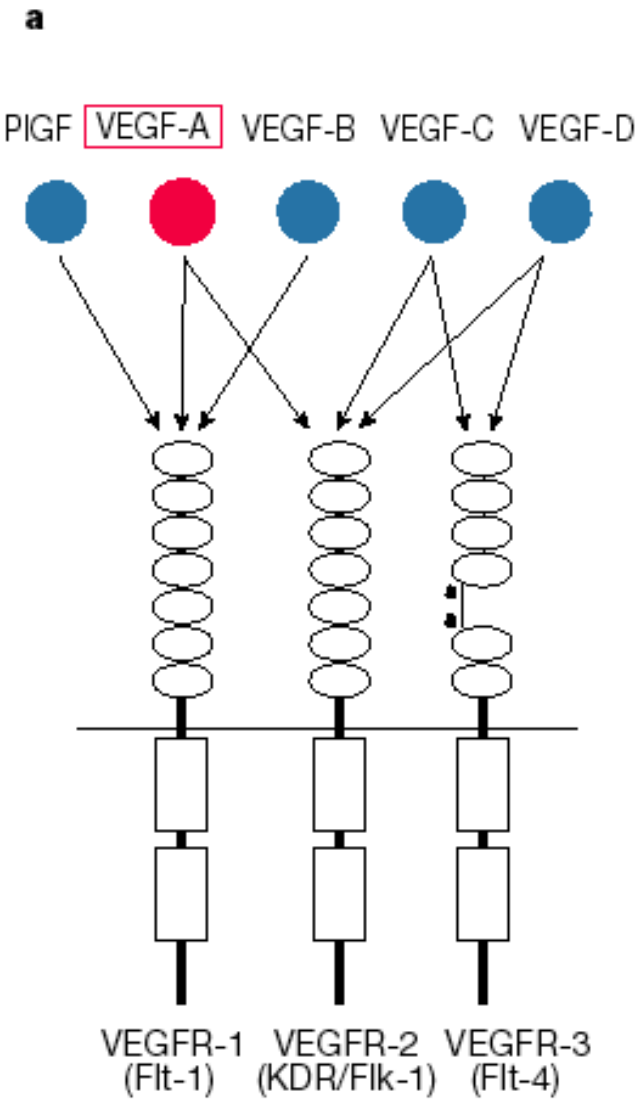
Mouse cornea: wounding induces angiogenesis, chemotactic response to angiogenic factors

Figure 22–27. Molecular Biology of the Cell, 4th Edition.

capillaries sprouting in
the retina of an embryonic mouse



VEGF/VEGFR family



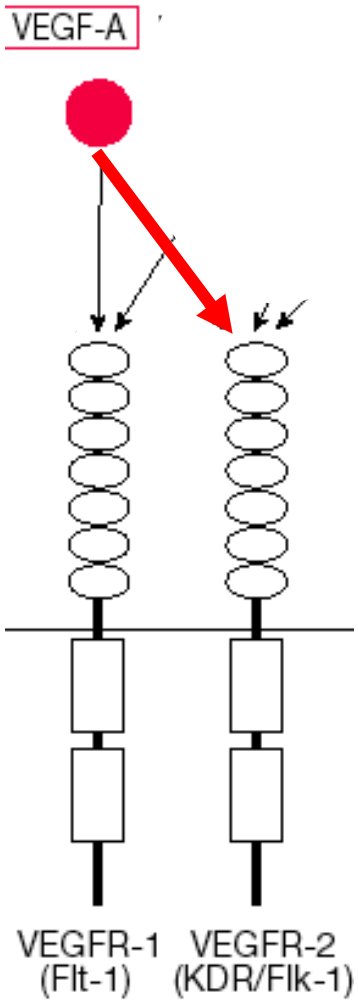
VEGF/VEGFR:

VEGF-A: initiation of vasculogenesis and sprouting angiogenesis,
Immature vessels,
Vascular permeability factor,
Haploid insufficiency in k.o. mice,

PlGF: remodeling of adult vessels
VEGF-B: heart vascularization ?
VEGF-C: lymphatic vessels
VEGF-D: lymphatic vessels ?

VEGFR-2: growth and permeability
VEGFR-1: negative role ?, decoy receptor, synergism with VEGFR-2 in tumor angiogenesis
VEGFR-3: lymphatic vessels

VEGF-A/VEGFR-2



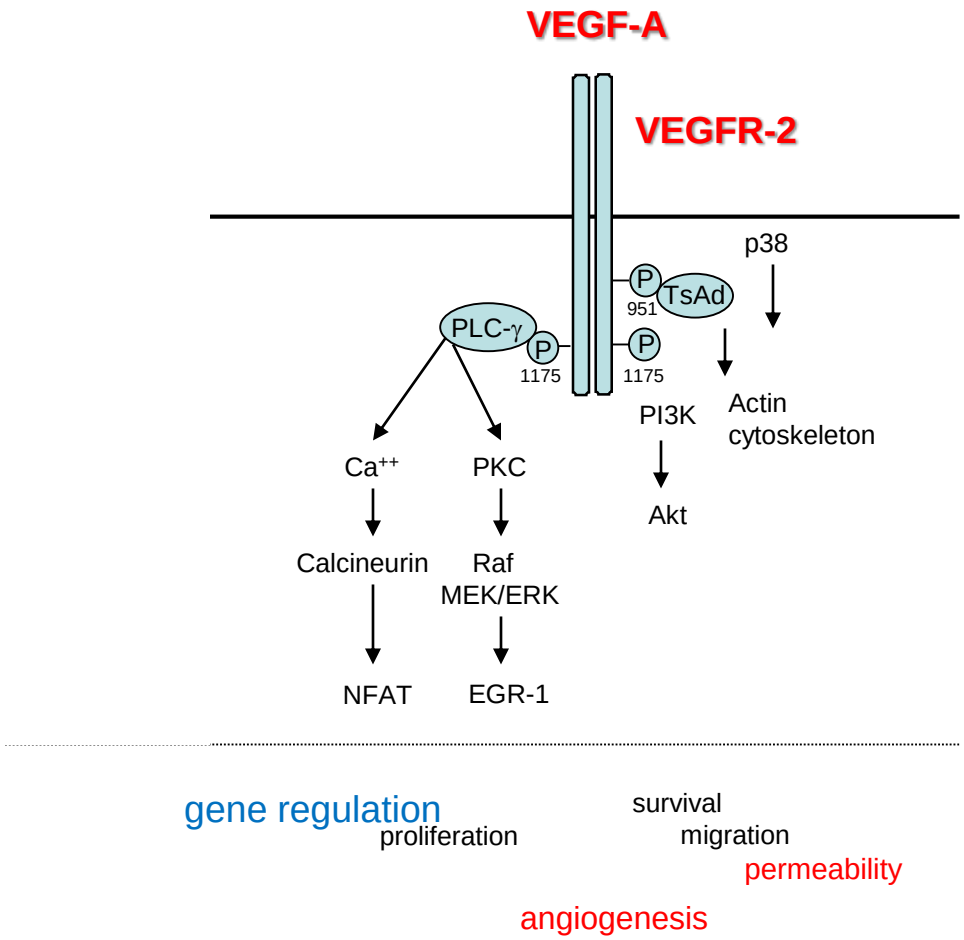
VEGF/VEGFR:

VEGF-A: initiation of vasculogenesis and sprouting angiogenesis,
Immature vessels,
Vascular permeability factor,

Haploid insufficiency in k.o. mice,

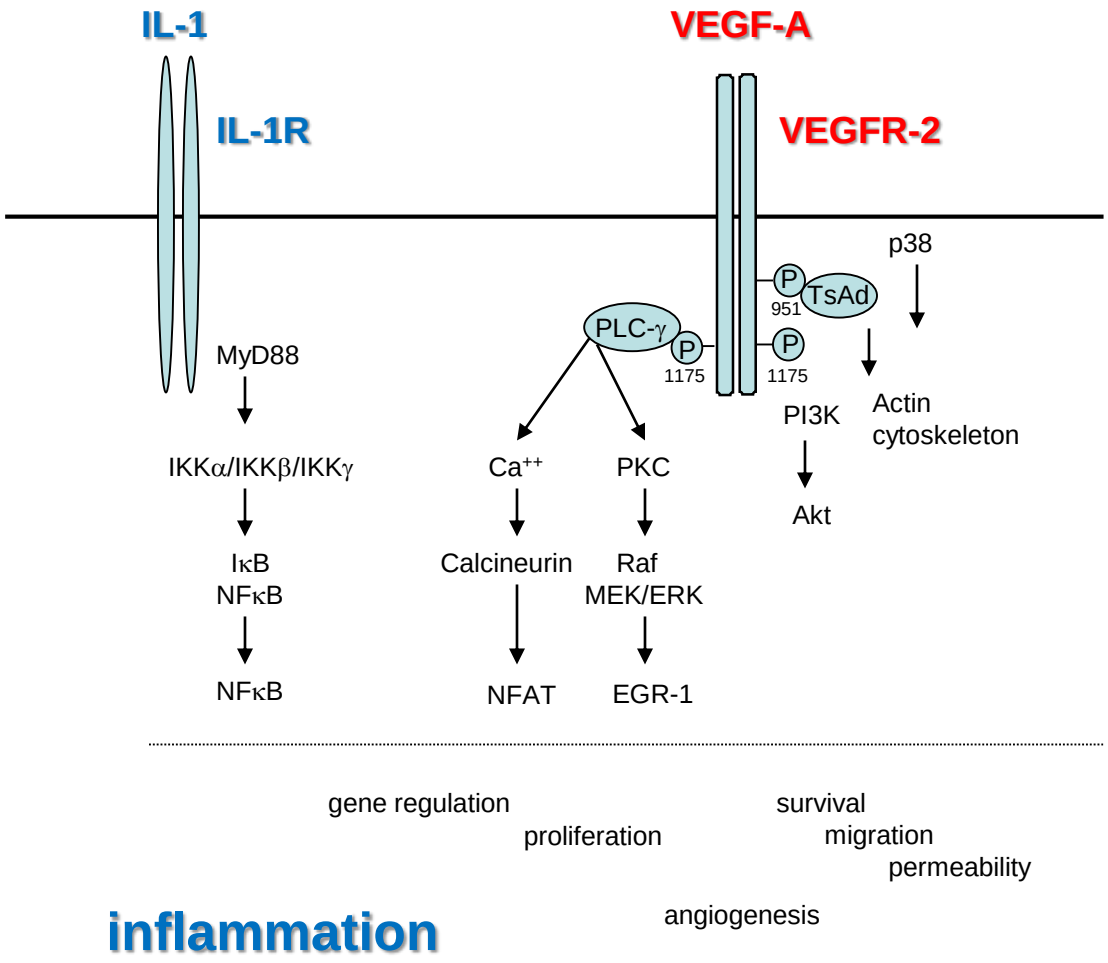
VEGFR-2: growth and permeability

Signaling by receptors of endothelial cells



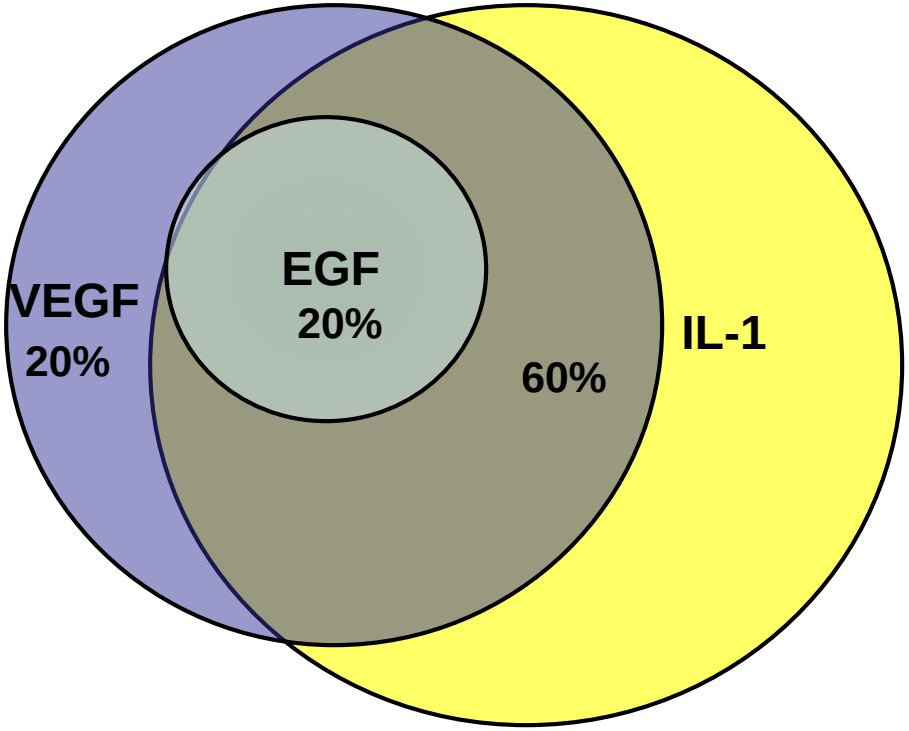
Hofer E.,Signaling transduction induced in endothelial cells by growth factor receptors involved in angiogenesis. Thrombosis ang haemostasis 2007

Signaling by receptors of endothelial cells



Hofer E.. Signaling transduction induced in endothelial cells by growth factor receptors involved in angiogenesis. Thrombosis ang haemostasis 2007

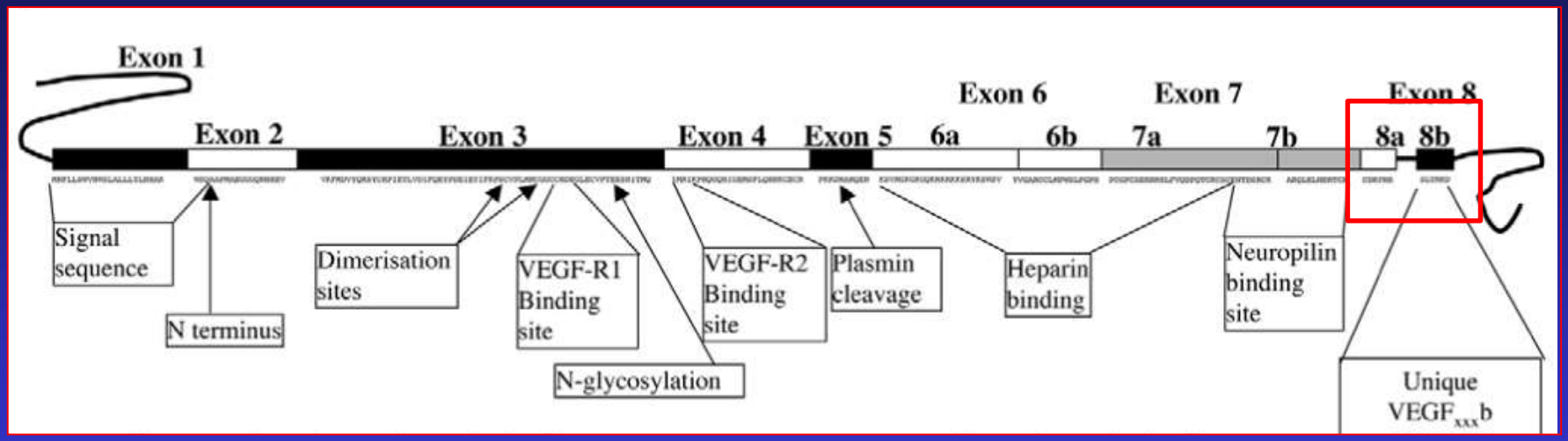
VEGF-induced genes overlap yuwith IL1-induced genes (50-60 %)

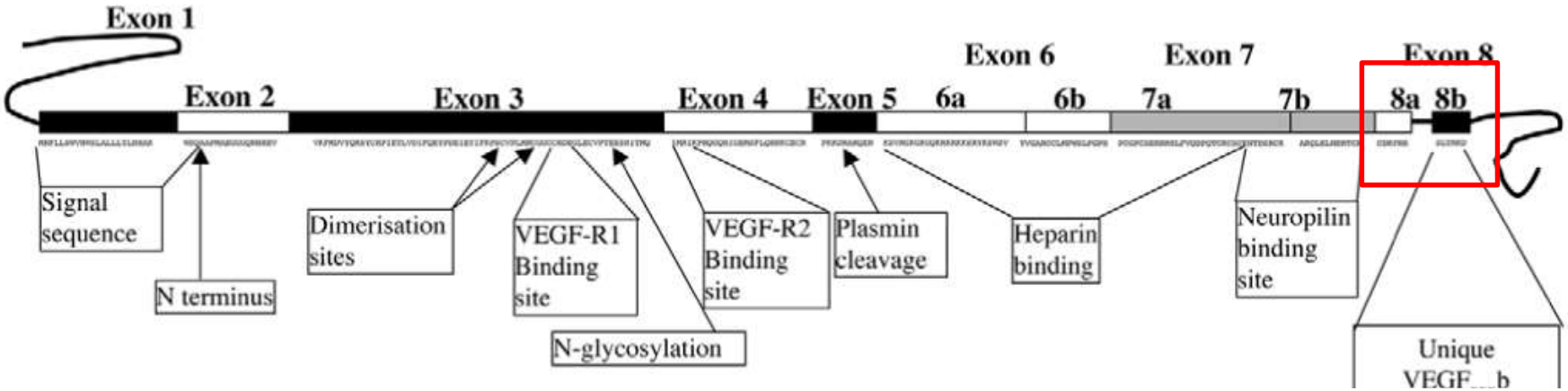


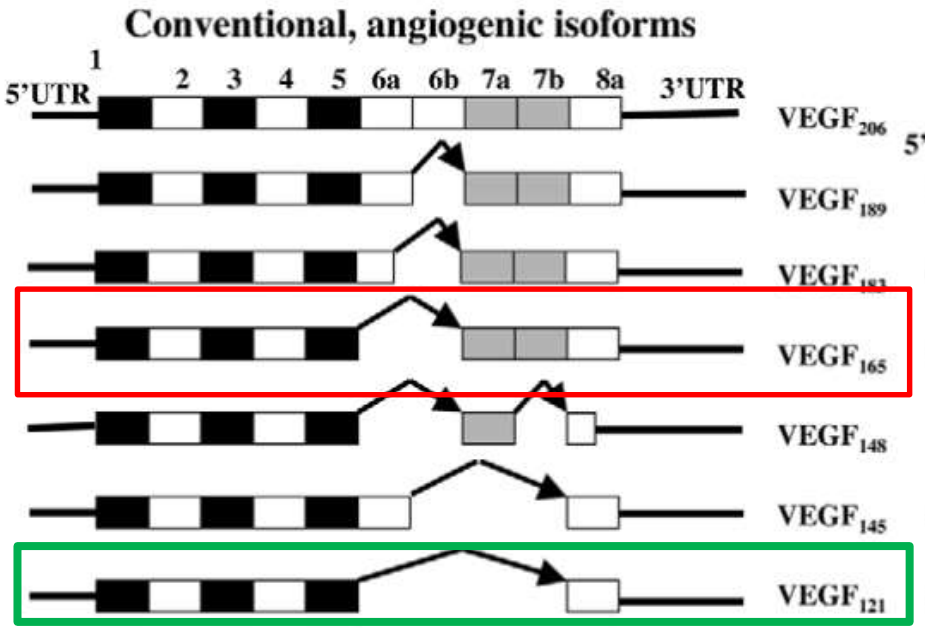
About 60 genes reproducibly induced by VEGF over 3-fold

Only 20 % of genes are preferentially induced by VEGF

VEGF Splicing and Isoforms

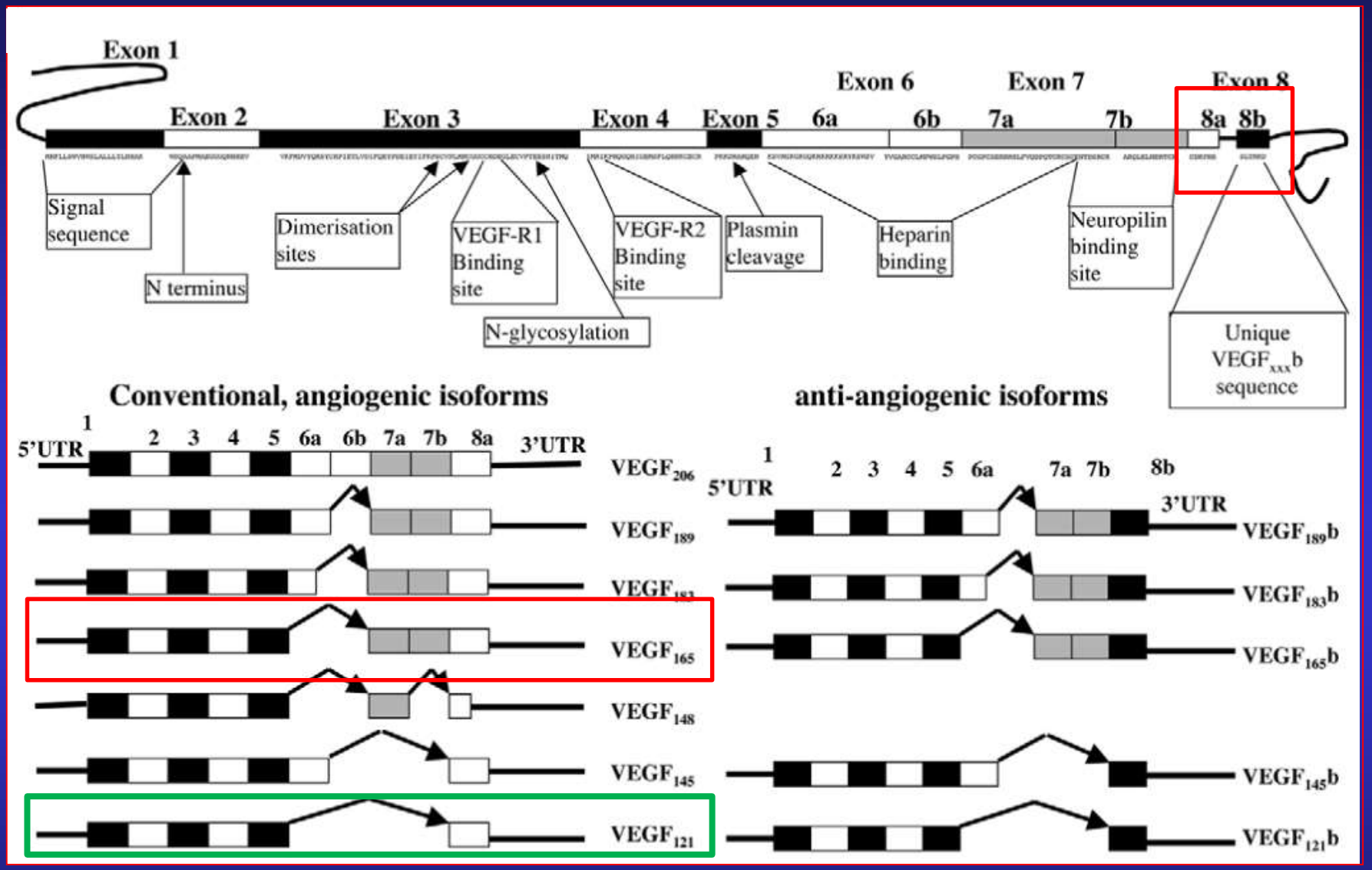

Exon 1
Exon 2
Exon 3
Exon 4
Exon 5
Exon 6
6a
6b
Exon 7
7a
7b
Exon 8
8a
8b
Signal sequence
N terminus
Dimerisation sites
VEGF-R1 Binding site
N-glycosylation
VEGF-R2 Binding site
Plasmin cleavage
Heparin binding
Neuropilin binding site
Unique VEGF$_{xxx}$b





VEFG165 vascolarizzazione patologica nell'occhio

VEGF121
Vascolarizzazione fisiologica


Exon 1
Exon 2
Exon 3
Exon 4
Exon 5
Exon 6
6a
6b
Exon 7
7a
7b
Exon 8
8a
8b
Signal sequence
N terminus
Dimerisation sites
VEGF-R1 Binding site
N-glycosylation
VEGF-R2 Binding site
Plasmin cleavage
Heparin binding
Neuropilin binding site
Unique VEGFxxxb sequence
Conventional, angiogenic isoforms
anti-angiogenic isoforms
5'UTR
1
2
3
4
5
6a
6b
7a
7b
8a
8b
3'UTR
VEGF206
VEGF189
VEGF183
VEGF165
VEGF148
VEGF145
VEGF121
VEGF189b
VEGF183b
VEGF165b
VEGF145b
VEGF121b

Le forme b

- VEGFxxxb family of isoforms are generated by the use of a more distal 3′ splice acceptor site

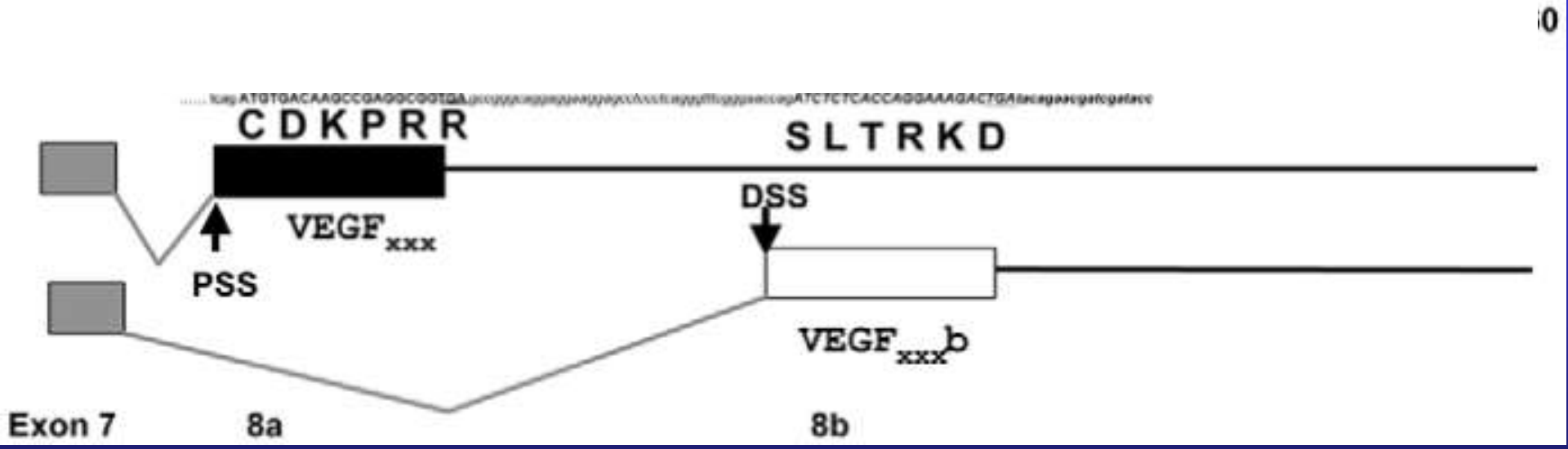
- Whereas the VEGFxxx isoforms (e.g. VEGF165) are **pro-angiogenic** and are upregulated in tumors, the VEGFxxxb isoforms (e.g. VEGF165b) **are anti-angiogenic** and downregulated in tumors.

- Anti-angiogenic activity is generated by **receptor binding but only weak receptor activation**, and inhibition of downstream VEGFR2 signalling

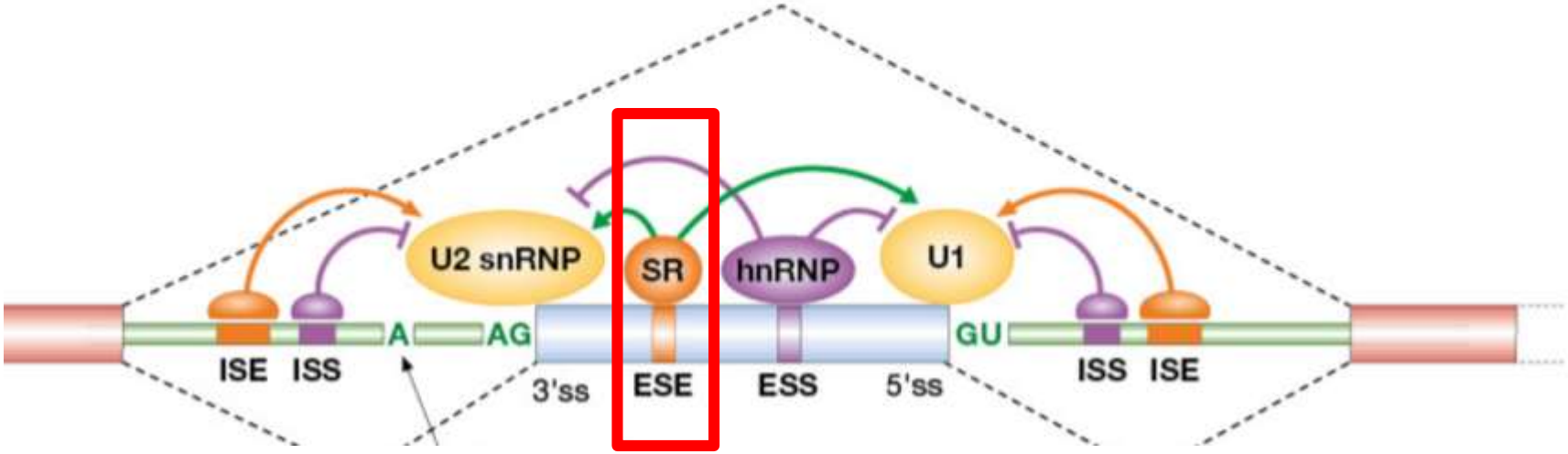
mRNA species that code for proteins of the same length
but with **different C-terminal six amino acids.**

Proximal splice-site selection (PSS)

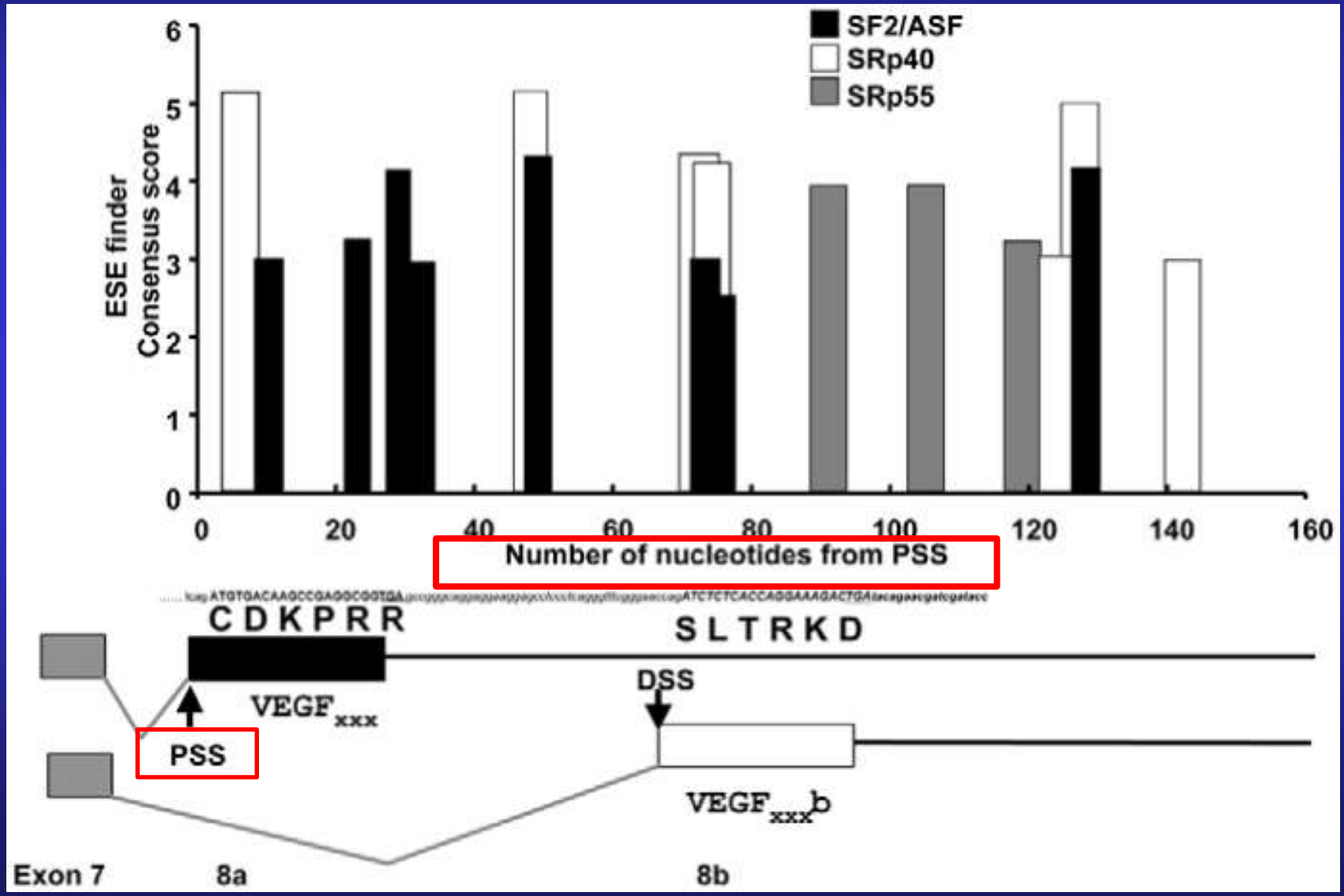
Distal splice-site selection DSS



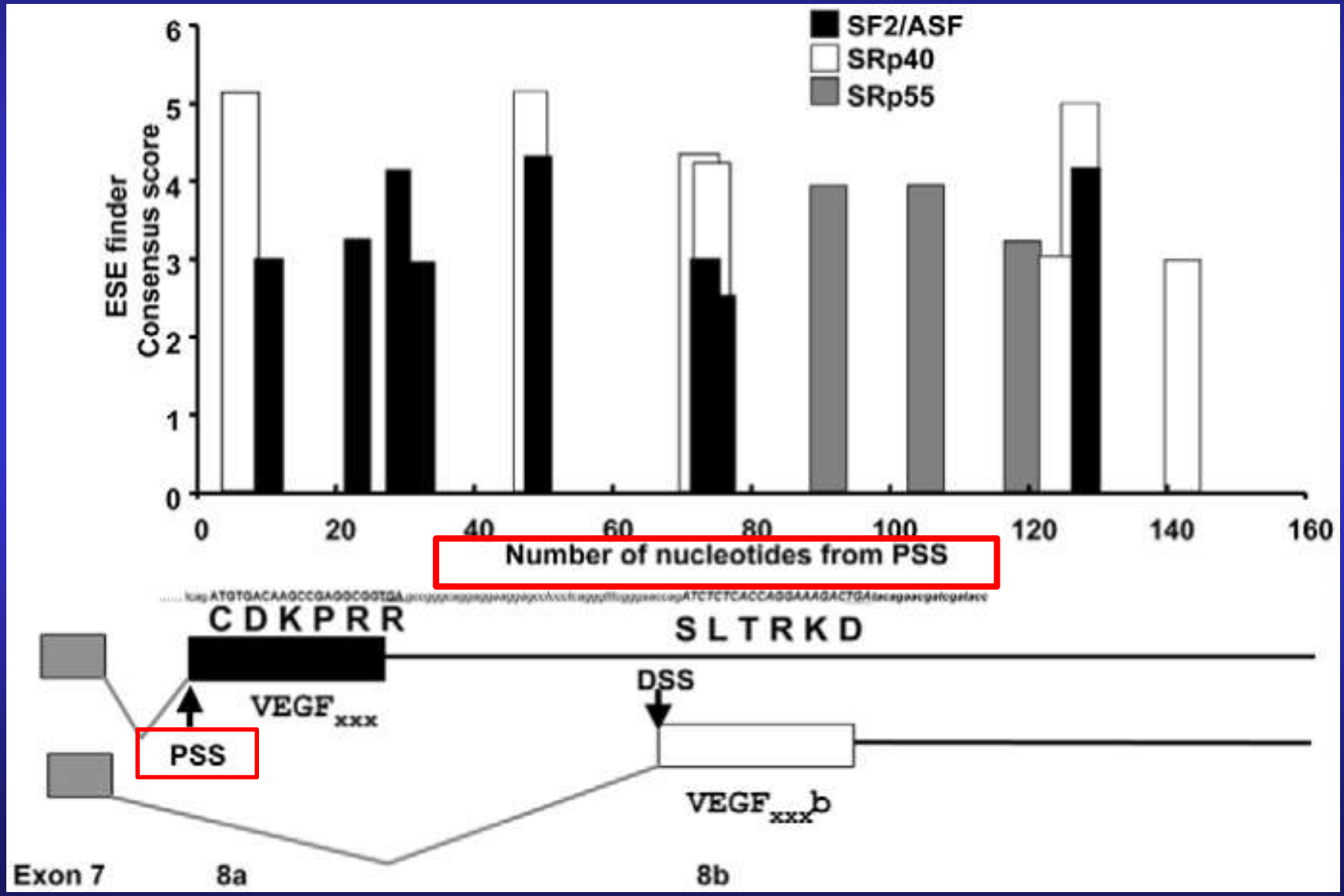
Regolazione dello splicing



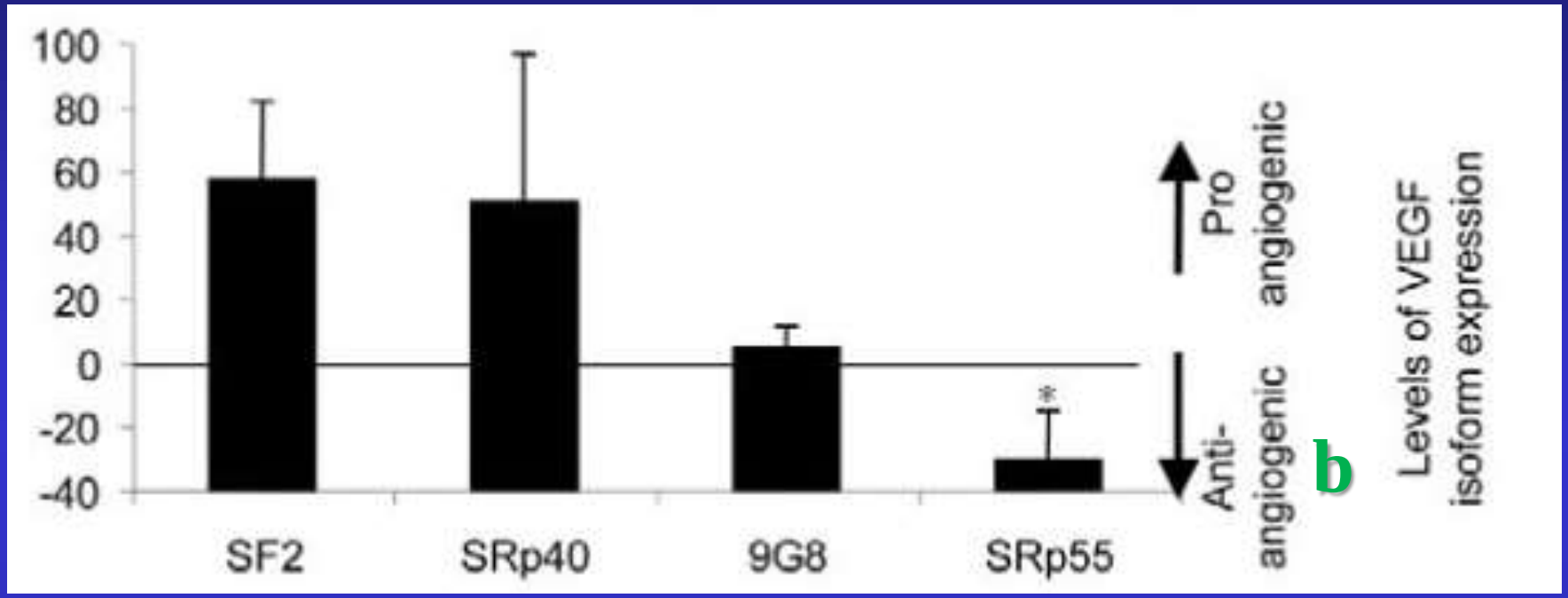
Distribution of ESE **Exonic Splicing Enhancers** consensus sequences in the C terminus of the VEGF gene.



The **SRp55** sites were associated with **distal splicing** whereas the SF2/ASF and SRp40 sites were associated with the **proximal splice**.



•Overexpression of splicing factors and VEGF isoform production - Retinal Cells



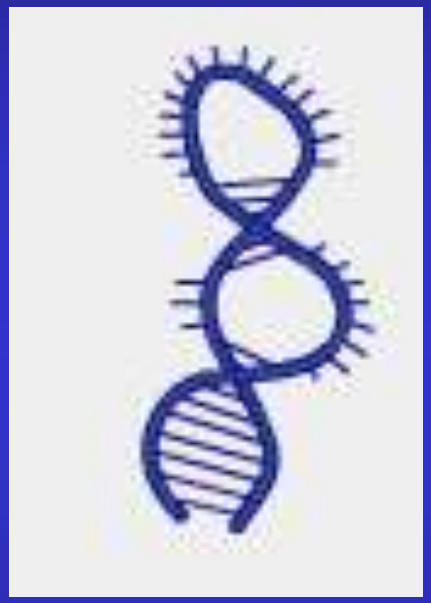
Solid line indicates an equal balance between the two sets of isoforms. Values below the line indicate anti-angiogenic balance

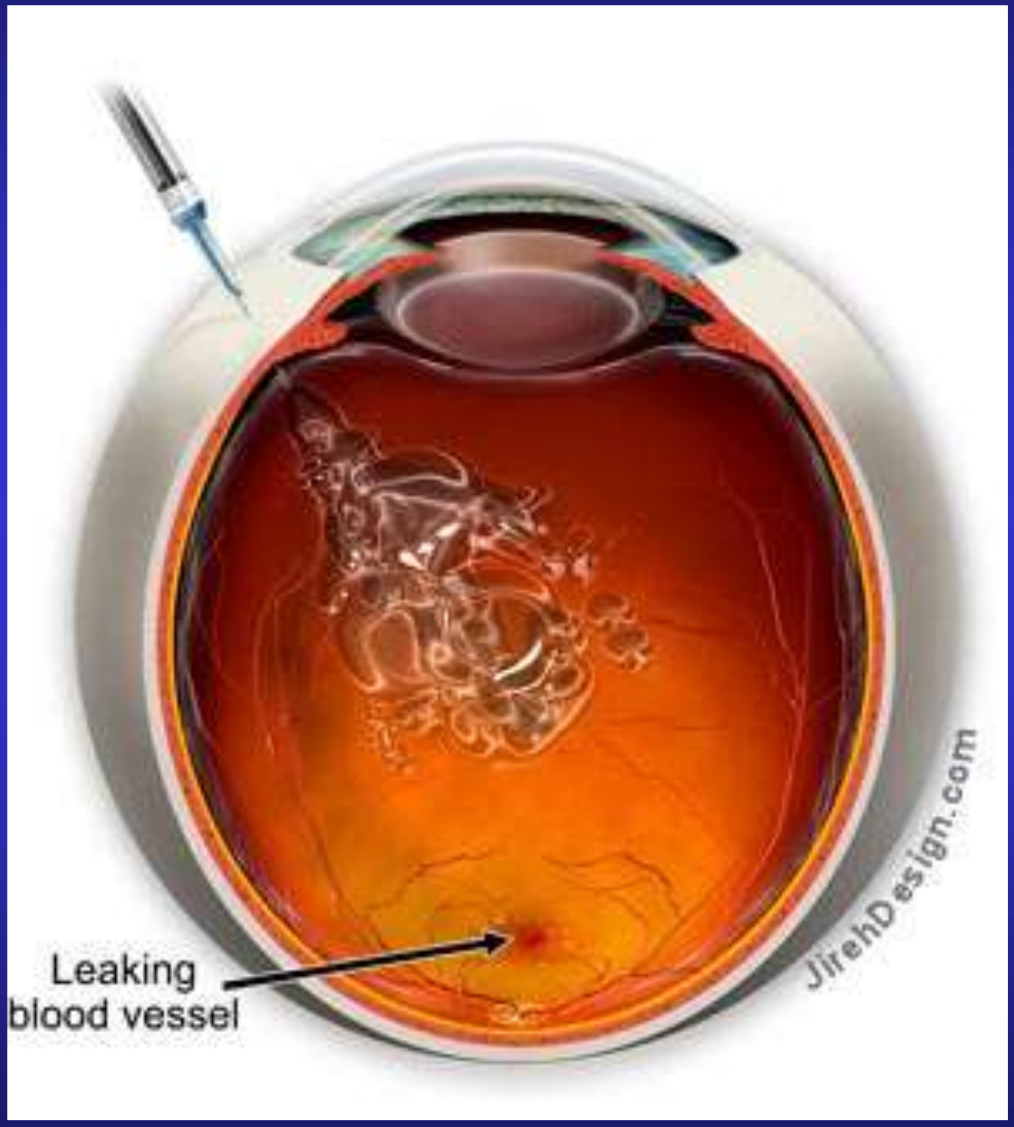
Vascular Endothelial Growth Factor and the Potential Therapeutic Use of Pegaptanib (Macugen®) in Diabetic Retinopathy

Starita C, Patel M, Katz B, Adamis A

Pegaptanib, an RNA aptamer used in the treatment of age related macular degeneration, binds and inactivates VEGF165.

In animal models it reverses the blood-retinal barrier breakdown.

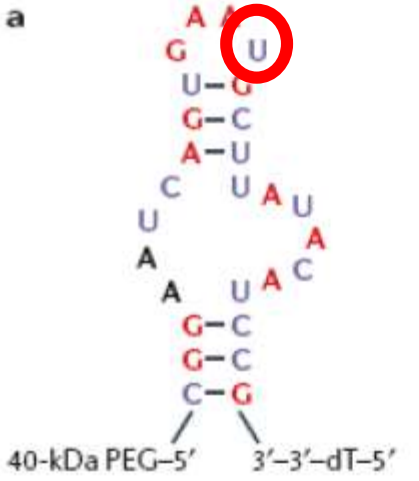



Leaking
blood vessel
JirehDesign.com

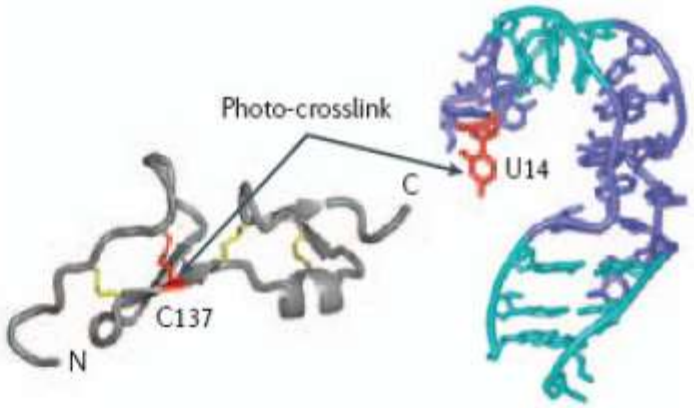
Il pegaptanib e` un antagonista selettivo del $VEGF_{165}$

Il Pegaptanib e` un aptamero a filamento singolo di **RNA** formato da 28 nucleotidi legato a 2 molecole di 20-kDa di glicole polietilenico (PEG)

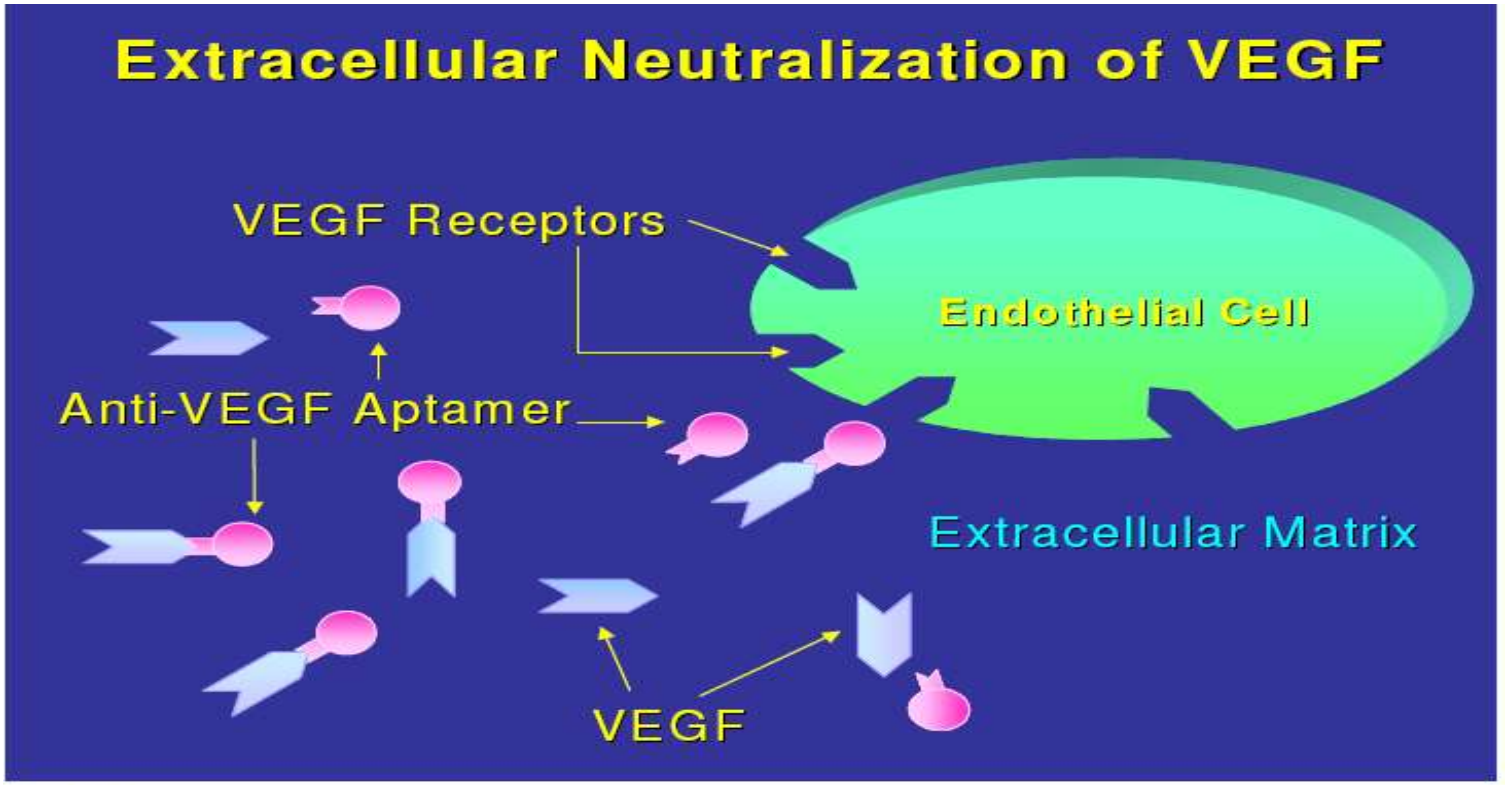
E` dotato di alta affinita` per il $VEGF_{165}$ (**vascolarizzazione patologica**) e nessun legame con il $VEGF_{121}$ (**vascolarizzazione fisiologica**)



a | Sequenza e struttura secondaria del pegaptanib.

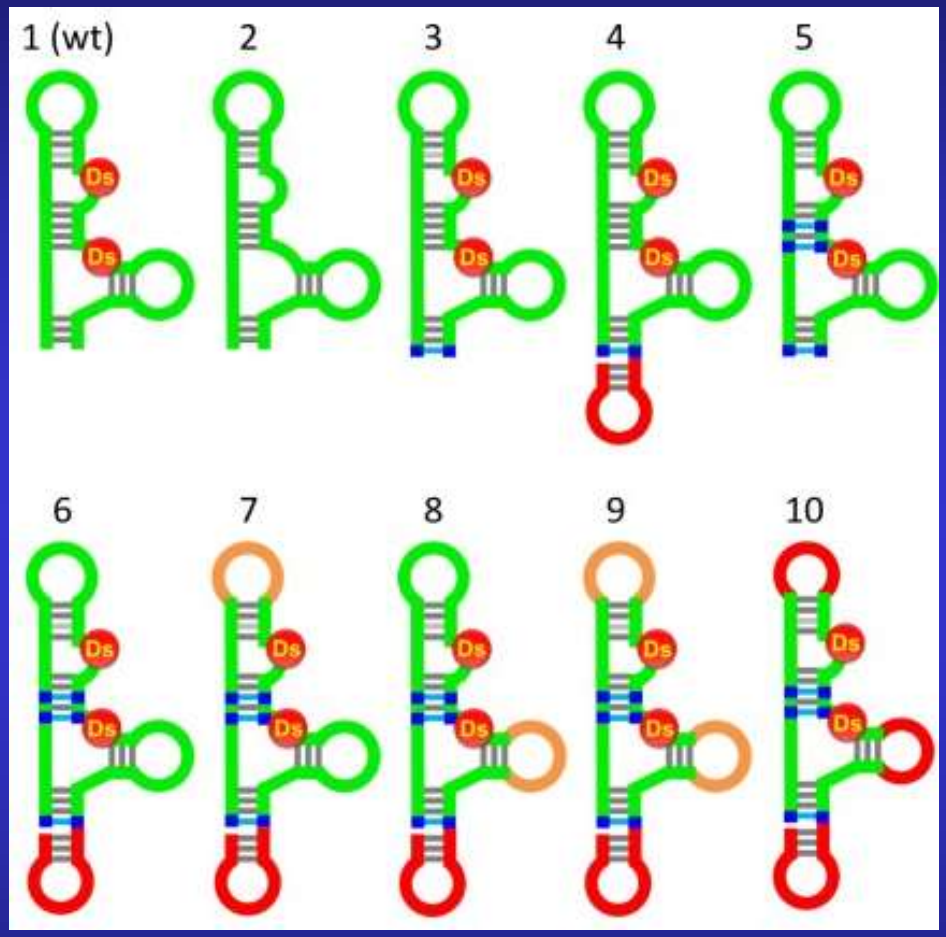


Il legame avviene tra la cisteina – 137 del $VEGF_{165}$ e l`uridina-14 dell`aptamero$_{14}$ (in rosso).



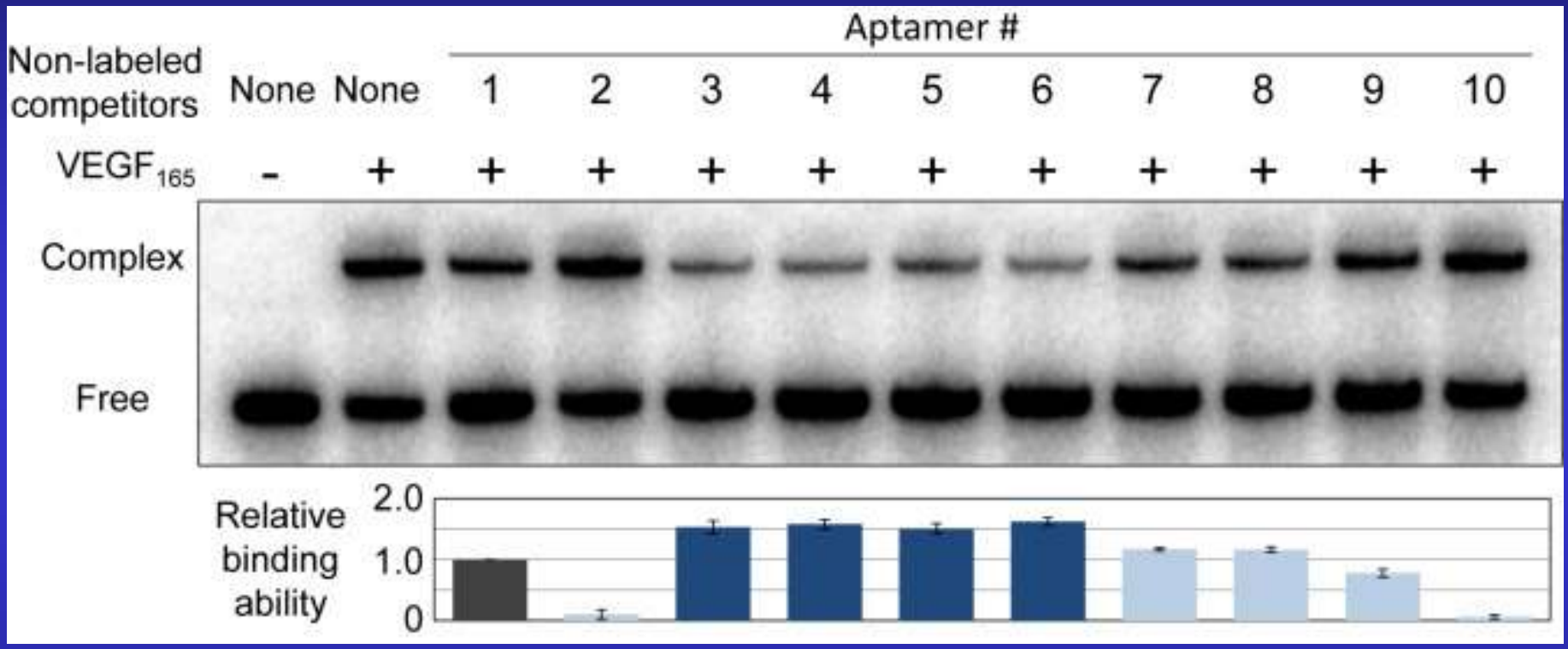
- Il Pegaptanib si lega specificamente al VEGF-165, impedendone l'aggancio con il suo recettore

Anti-VEGF165 DNA aptamer variants



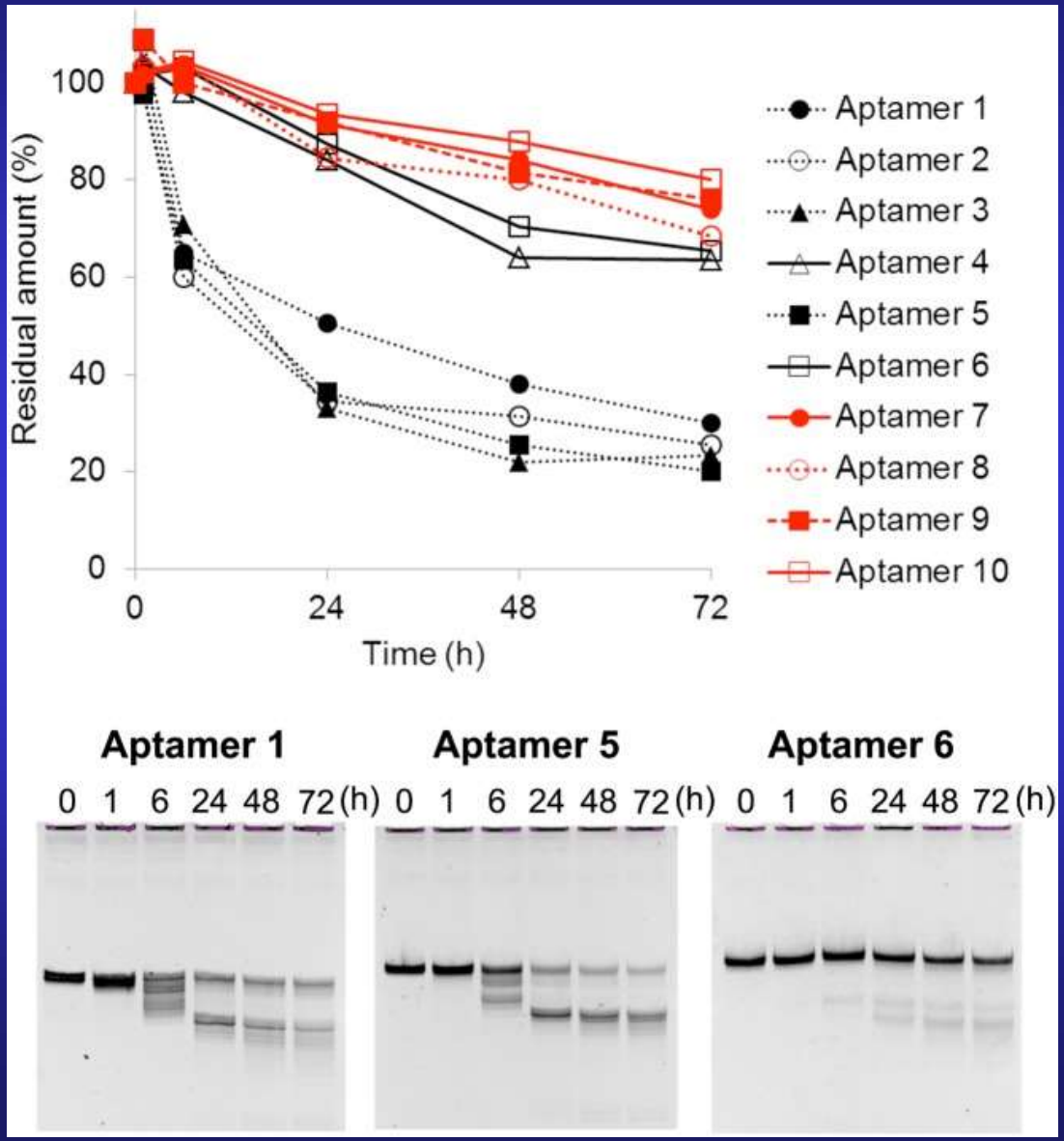
Ds Unnatural Base

Aptamer VEGF165 complex formation competition assay

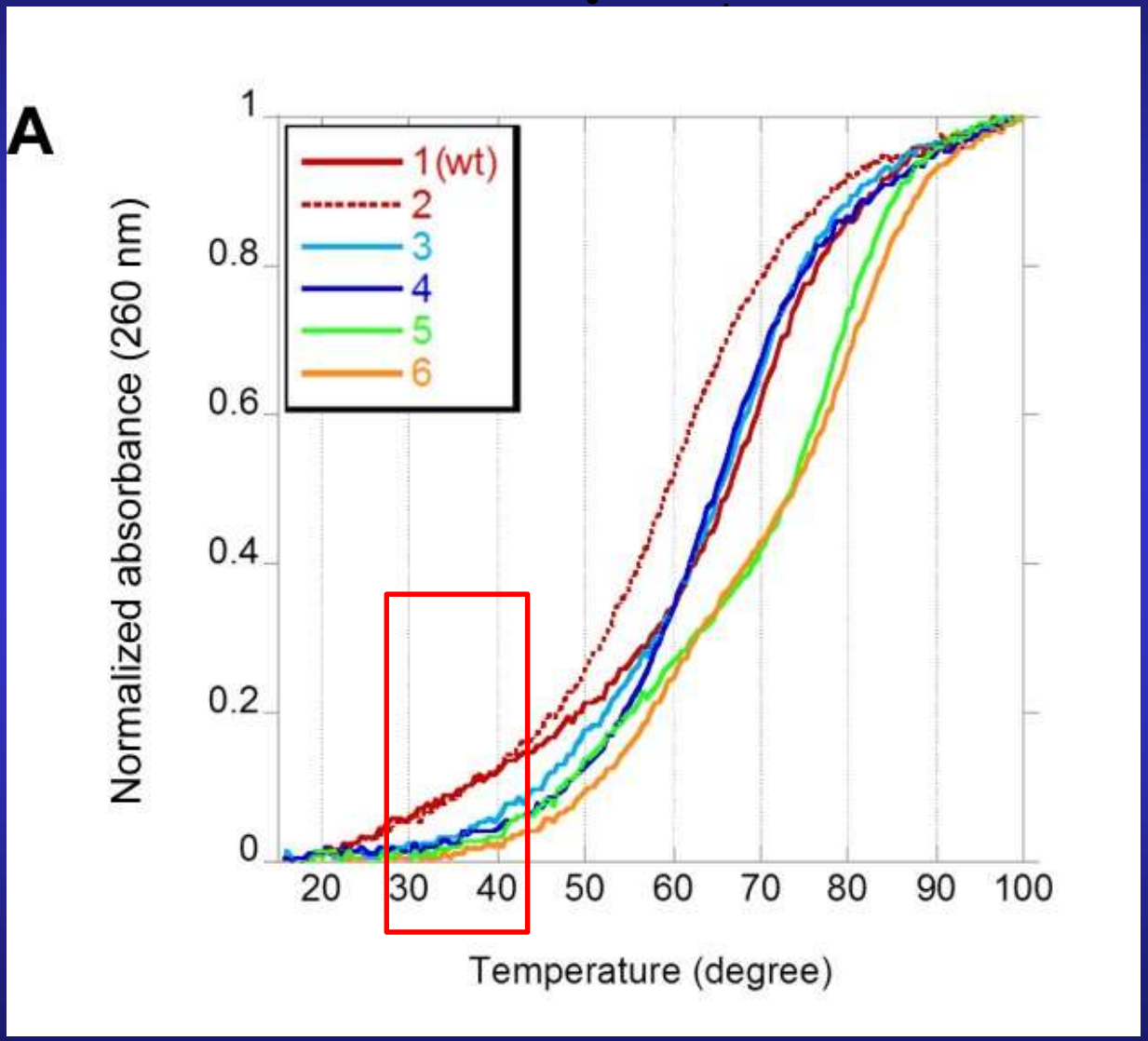


32P-labeled Aptamer 1 (100 nM) was incubated with VEGF165 (100 nM), in the presence of each non-labeled variant as a competitor (100 nM), at 37°C for 30 min

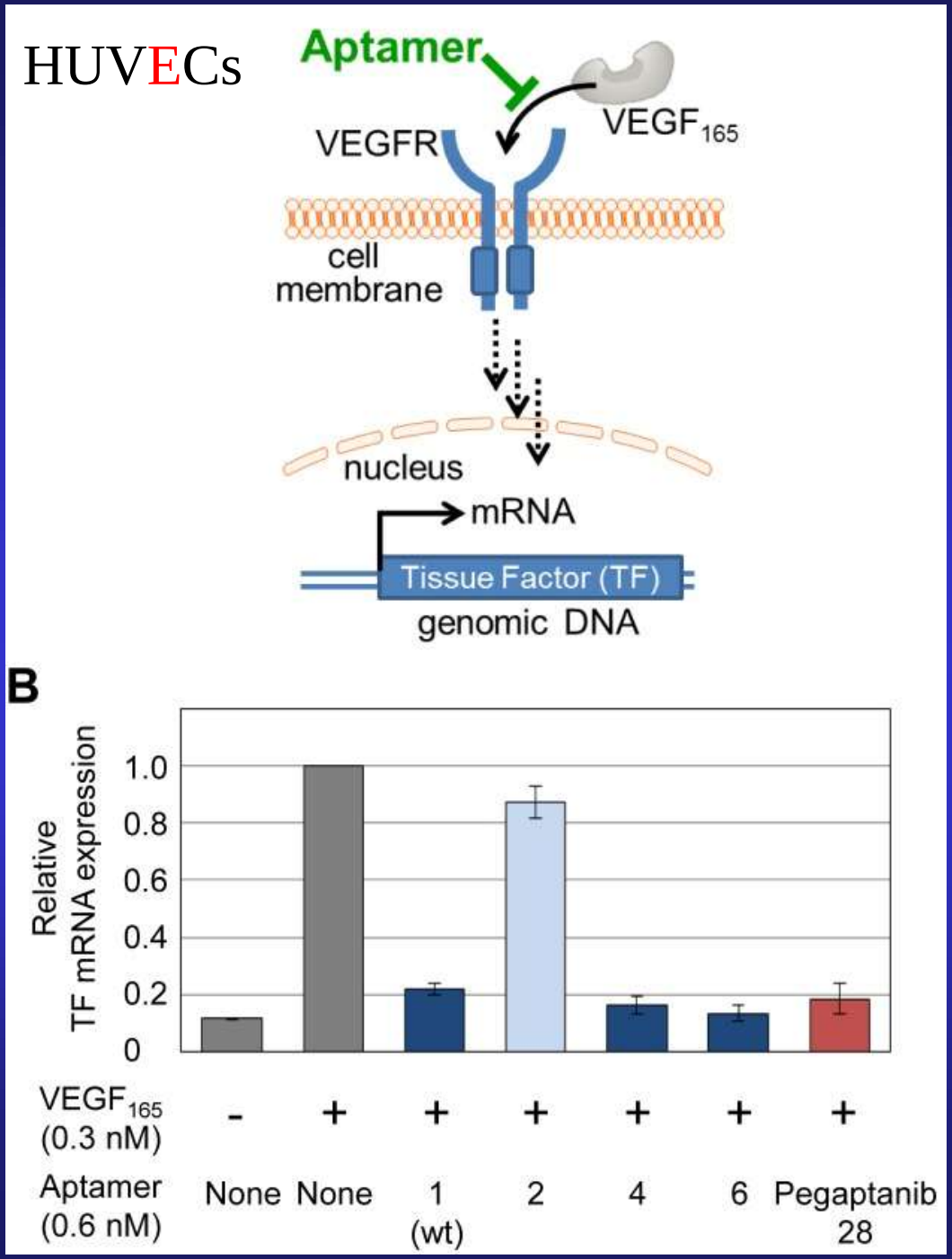
Nuclease resistance of anti-VEGF165 aptamers in human serum

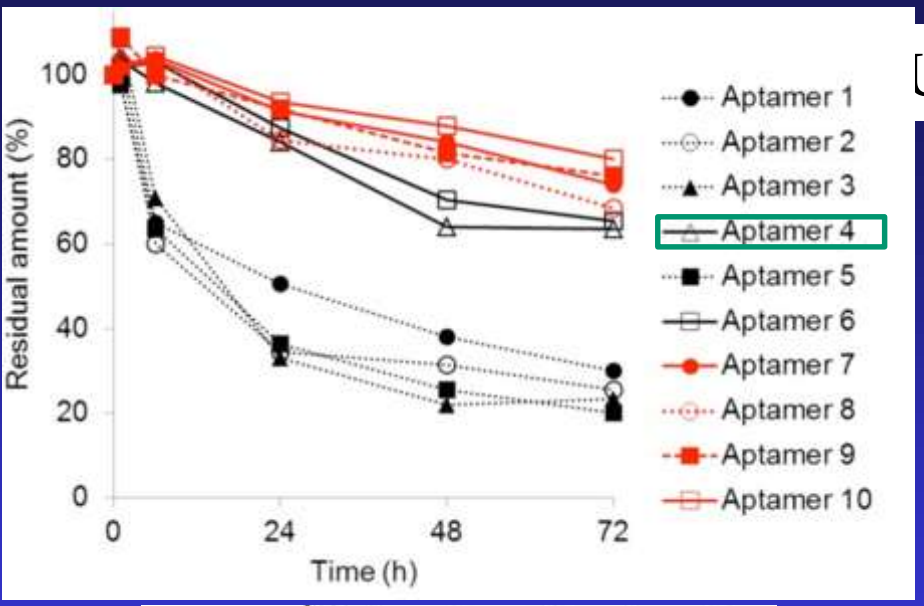


Thermal stabilities of aptamers

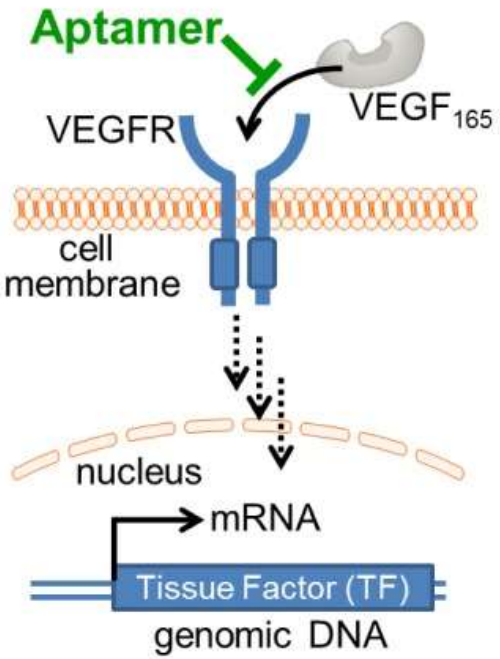


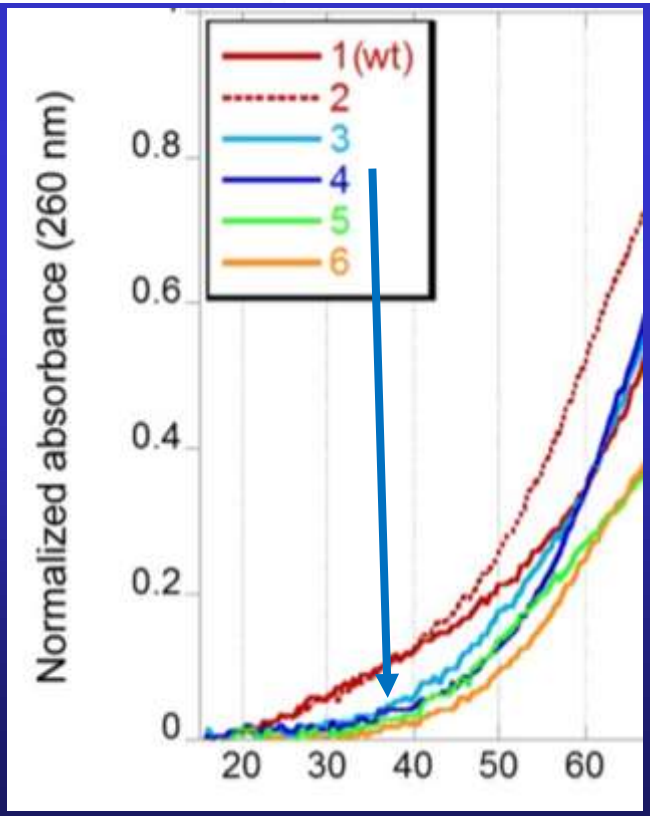
Inhibition of the interaction between VEGF165 and its receptor by aptamers



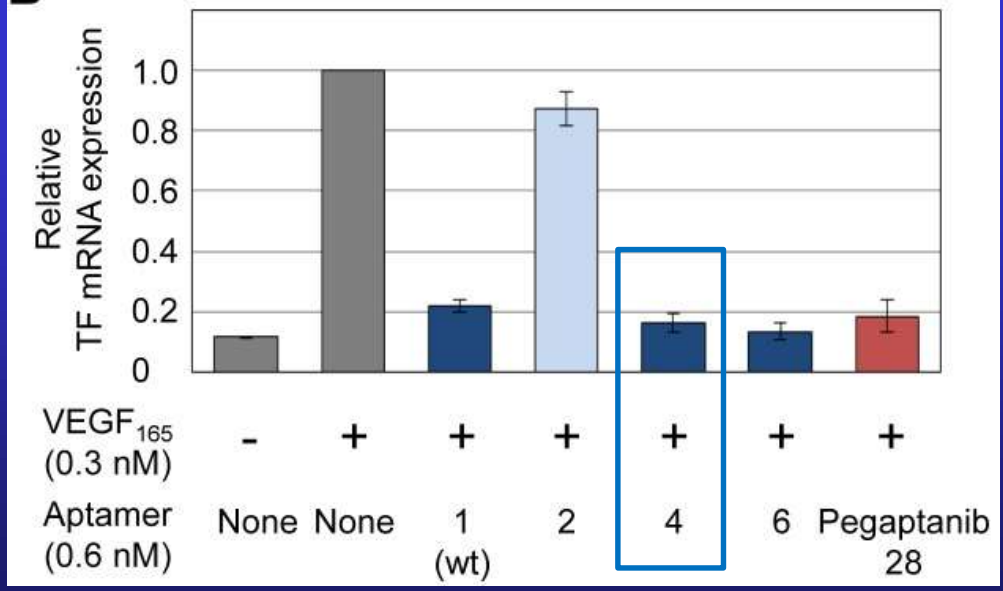

Residual amount (%)
100
80
60
40
20
0
0
24
48
72
Time (h)
Aptamer 1
Aptamer 2
Aptamer 3
Aptamer 4
Aptamer 5
Aptamer 6
Aptamer 7
Aptamer 8
Aptamer 9
Aptamer 10

UVECs


Aptamer
VEGF165
VEGFR
cell membrane
nucleus
mRNA
Tissue Factor (TF)
genomic DNA


Normalized absorbance (260 nm)
0.8
0.6
0.4
0.2
0
20
30
40
50
60
1 (wt)
2
3
4
5
6

B


Relative TF mRNA expression
1.0
0.8
0.6
0.4
0.2
0
VEGF165 (0.3 nM)
- + + + + + +
Aptamer (0.6 nM)
None None 1 (wt) 2 4 6 Pegaptanib 28

CONCLUSIONI

Aptameri diretti contro specifiche isoforme di VEGF contrastano la degenerazione senile della Macula