Omic Approaches in Biomarker Discovery: New Perspectives in Cancer Diagnostics and Therapy

Strategies and approaches in looking for diagnostic / prognostic cancer markers

Before-genomic *versus* post-genomic era

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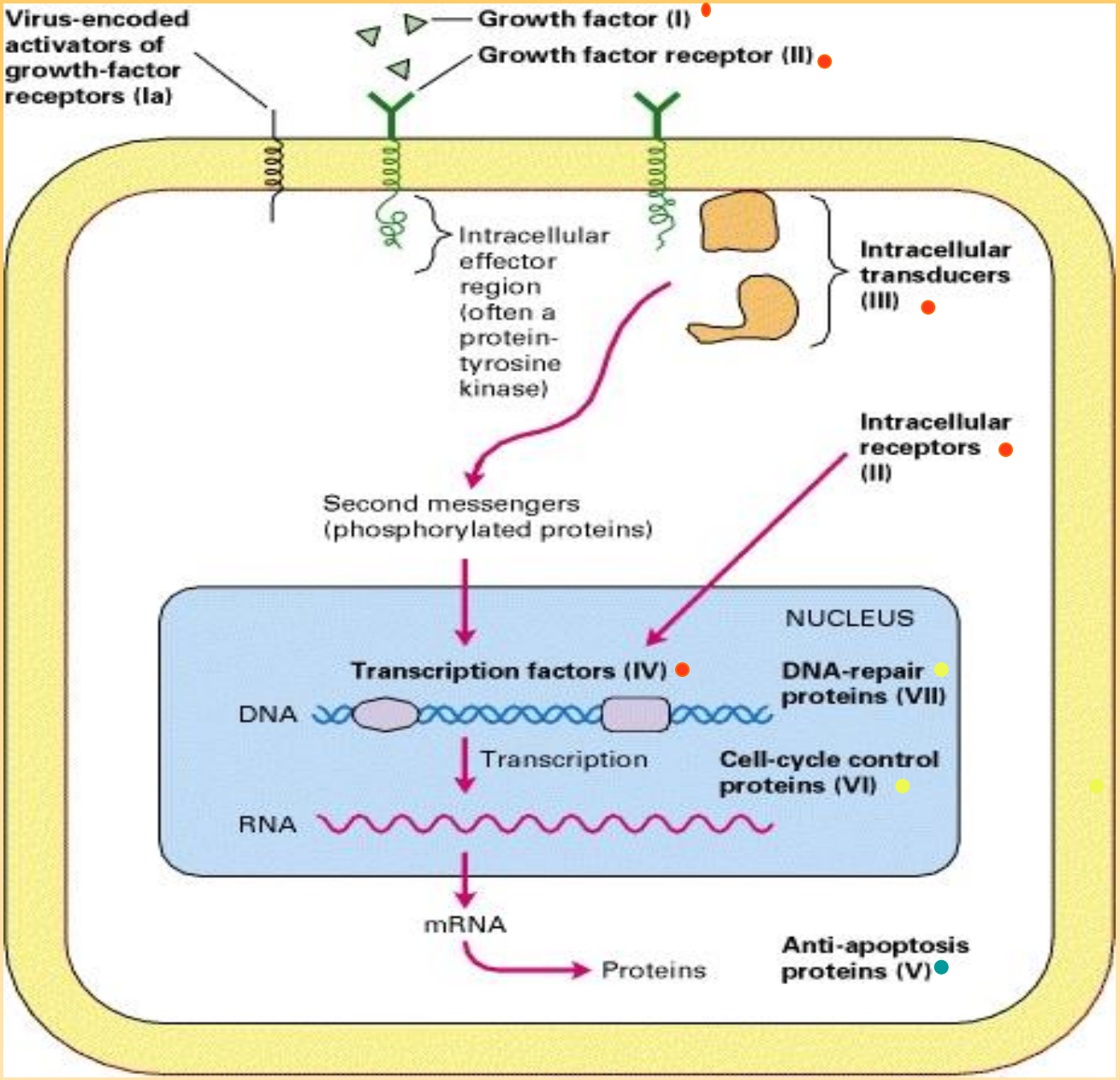
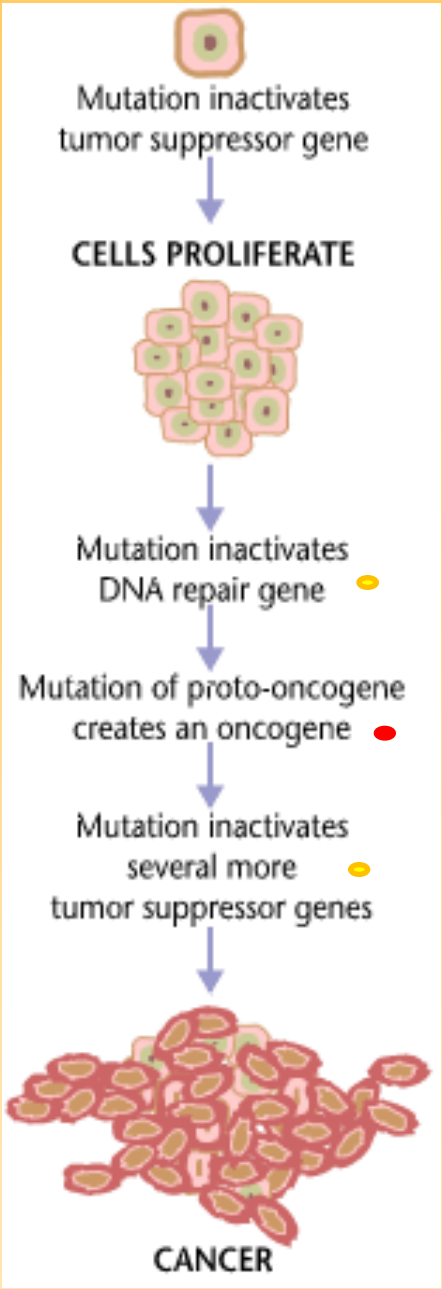


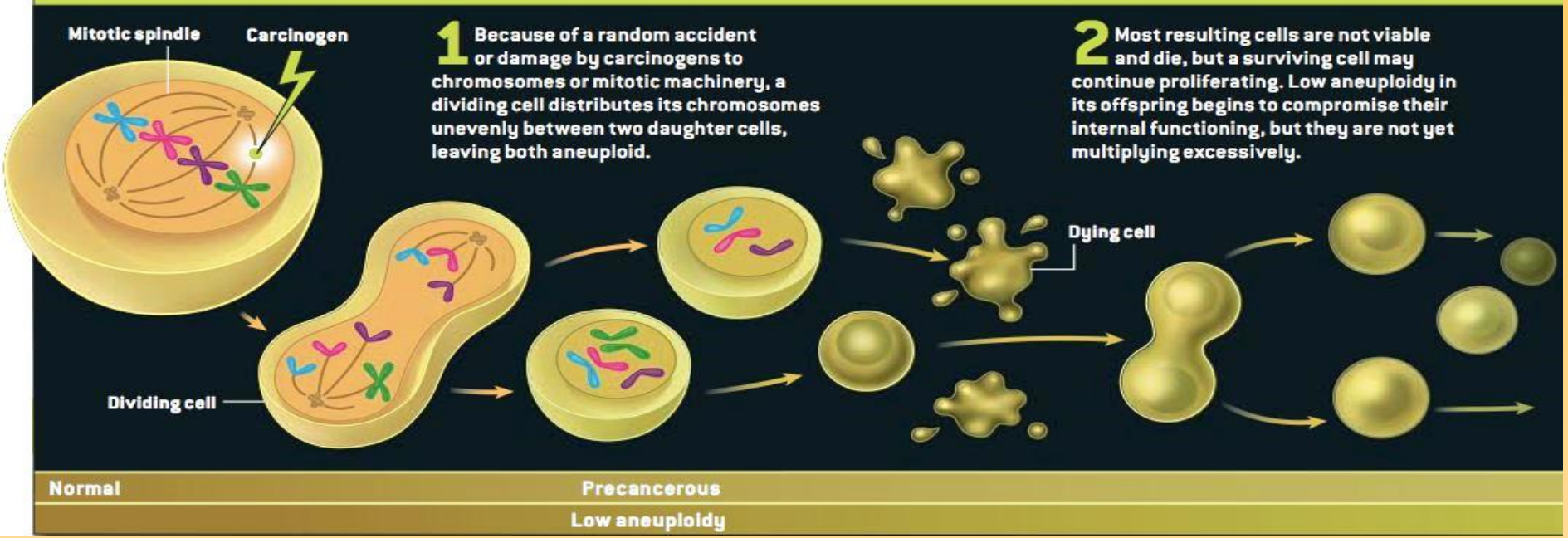


Klasična hipoteza o nastanku raka: (proto)onkogeni in tumorje zaviralni (supresorski) geni.

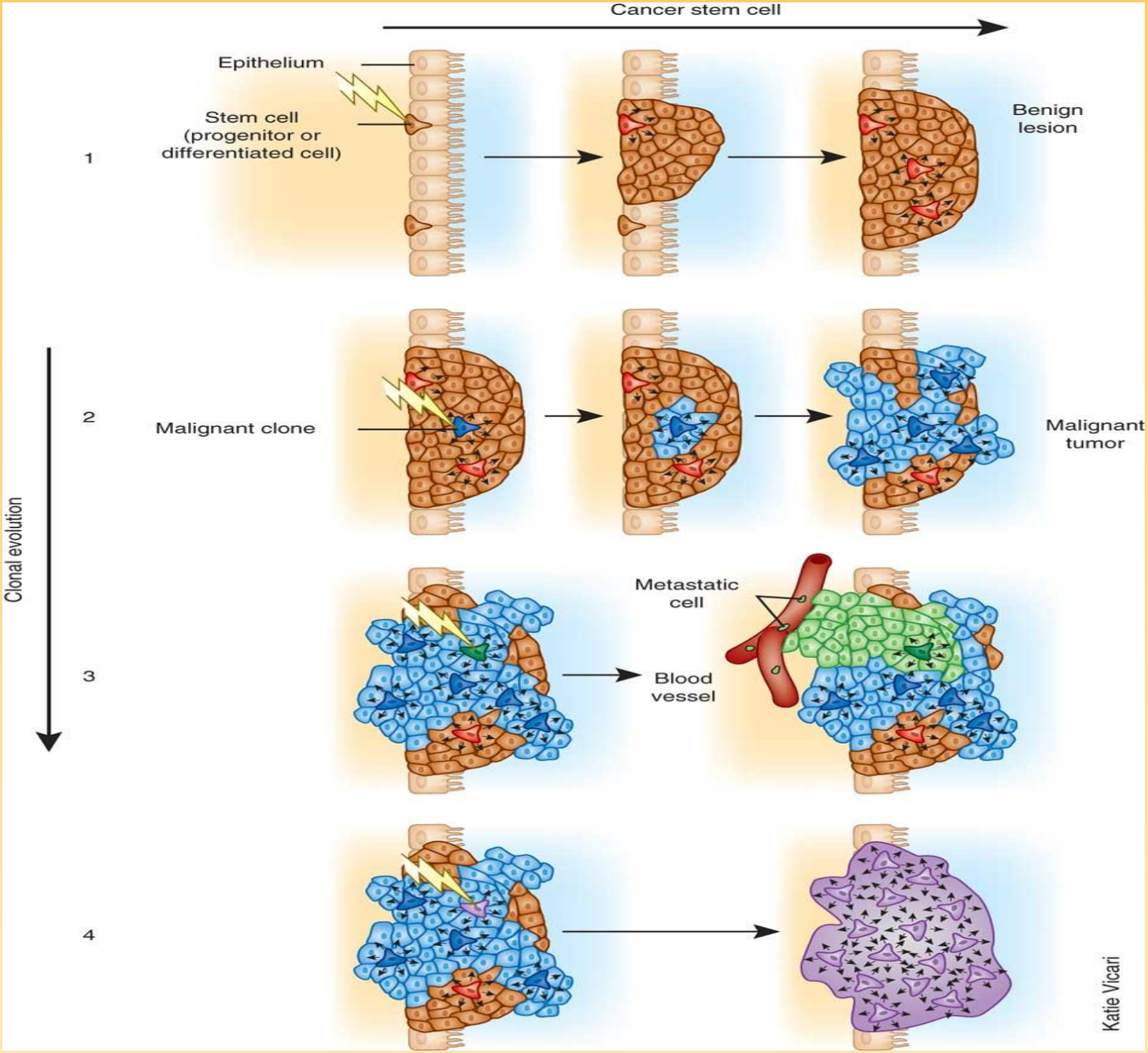
http://en.wikipedia.org/wiki/Cancer

HOW ANEUPLOIDY COULD CAUSE CANCER

Abnormal chromosome numbers in a cell create conditions that lead to further chromosome damage and disarray. With each new generation, resulting cells grow increasingly unstable and develop ever more malignant traits.

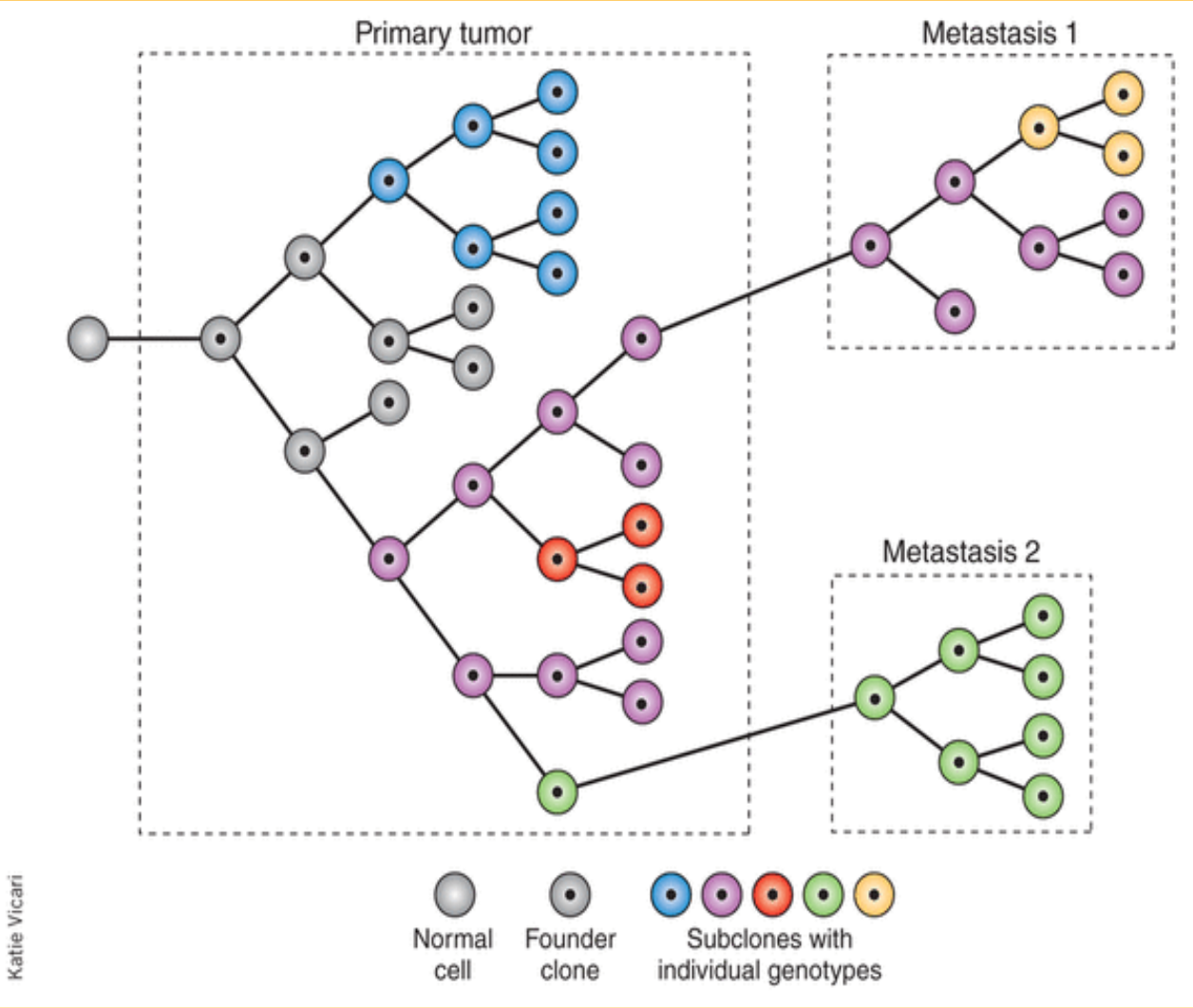


Hipoteza o anevploidnosti kot primarnem vzroku za nastanek raka.



Klonski razvoj raka.

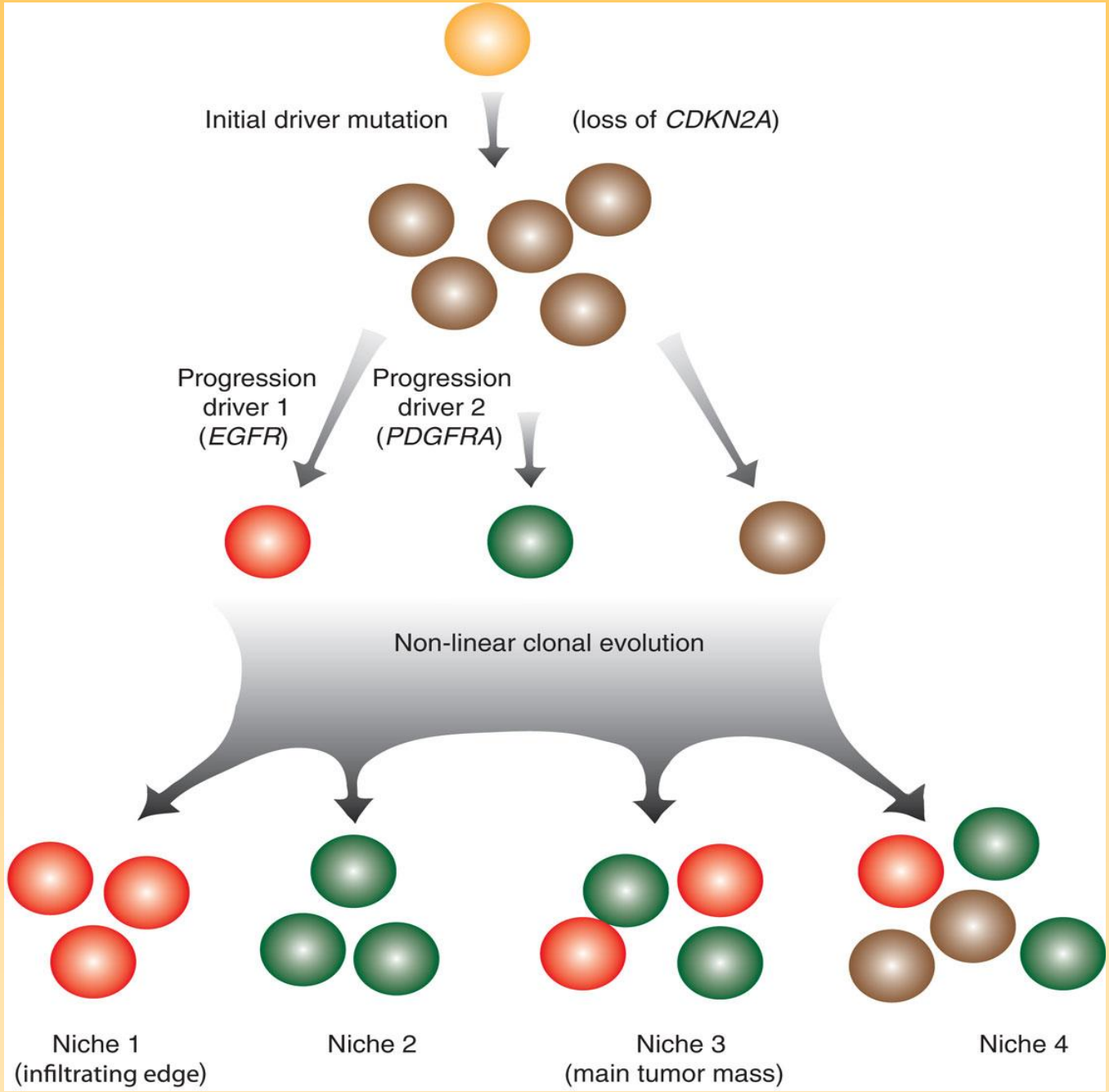
H. Clevers: *Nature Medicine*, 2011, **13**:141.



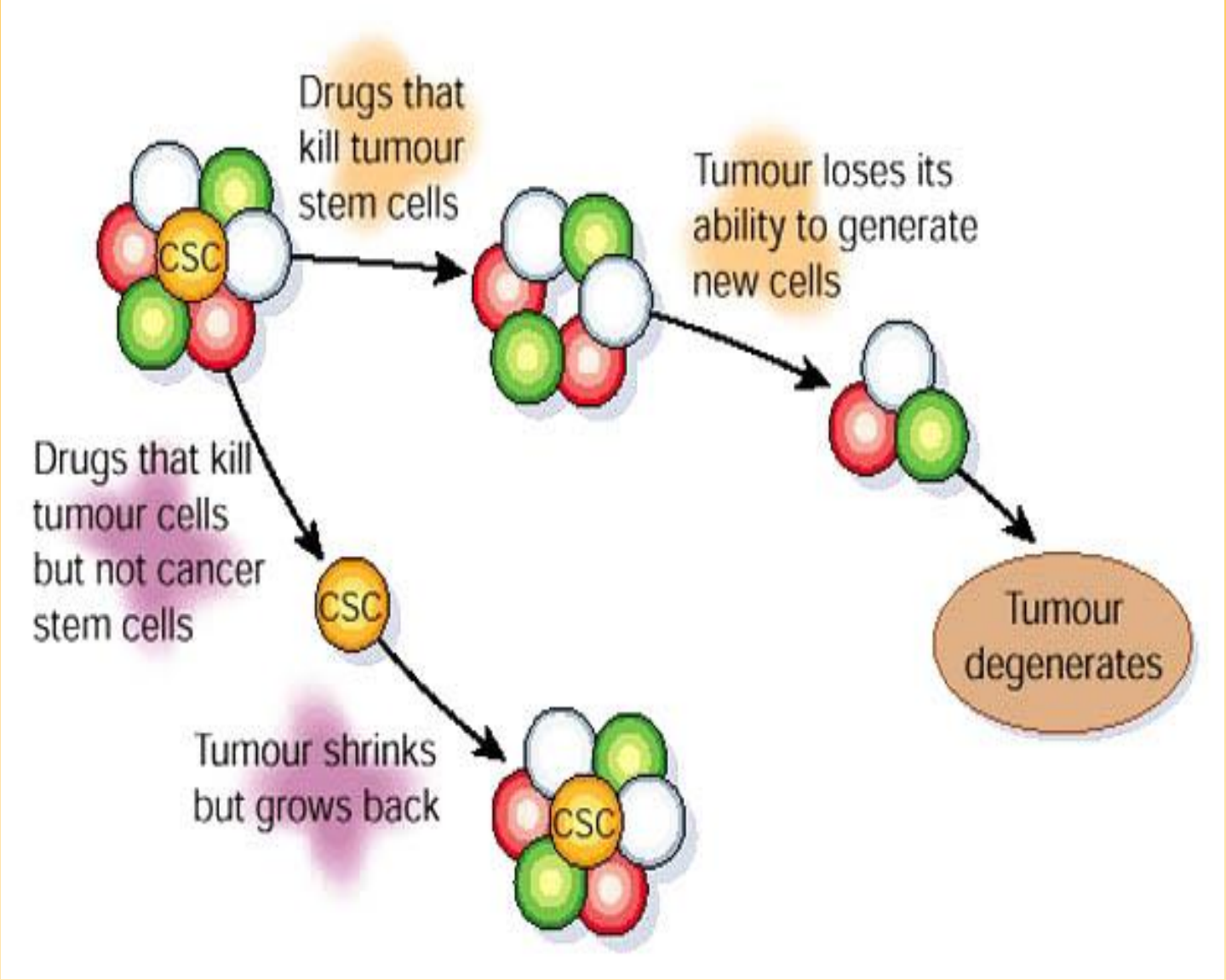
Pri klonskem razvoju raka nastanejo subpopulacije različno malignih celic.

C. Caldas: *Nature Biotechnol*., 2012. **13**:141.

Različni predeli tumorja so lahko naseljeni z več vrstami celic različne stopnje malignosti.



Chen and Ding: *Genome Biology*, 2012, **13**:141.

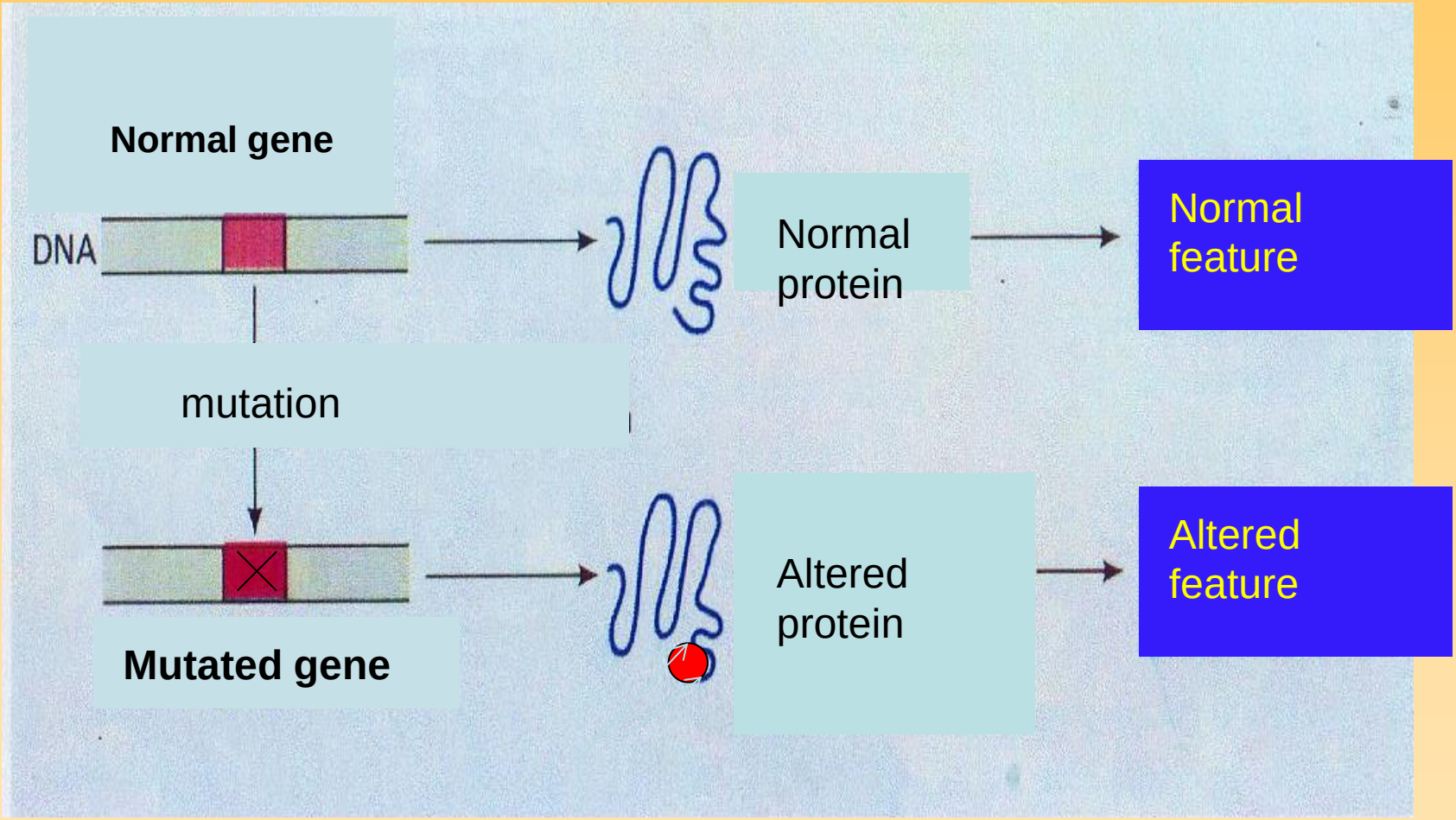


Hipoteza o matičnih celicah raka.

T. Reya et al.: *Nature,* 2001, **414**:105-111.

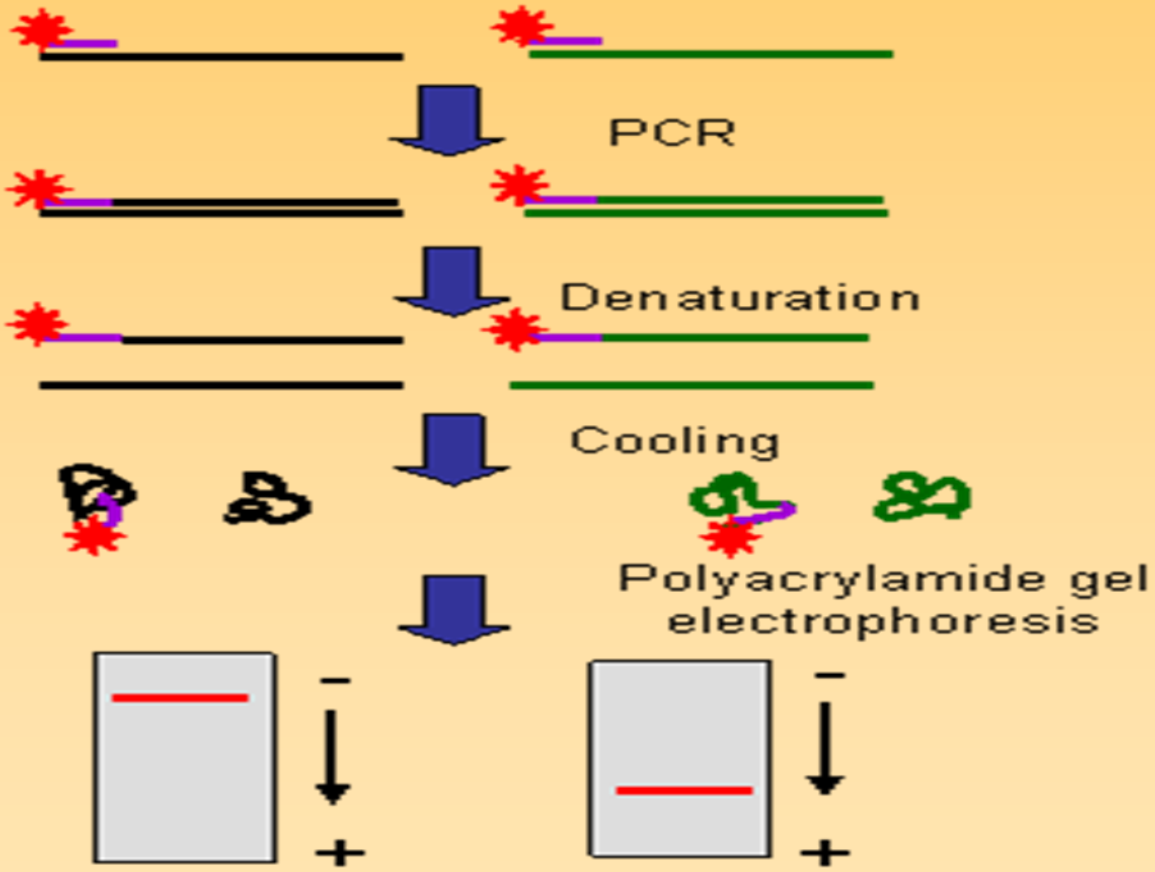
»Pre-GENOMIC« ERA

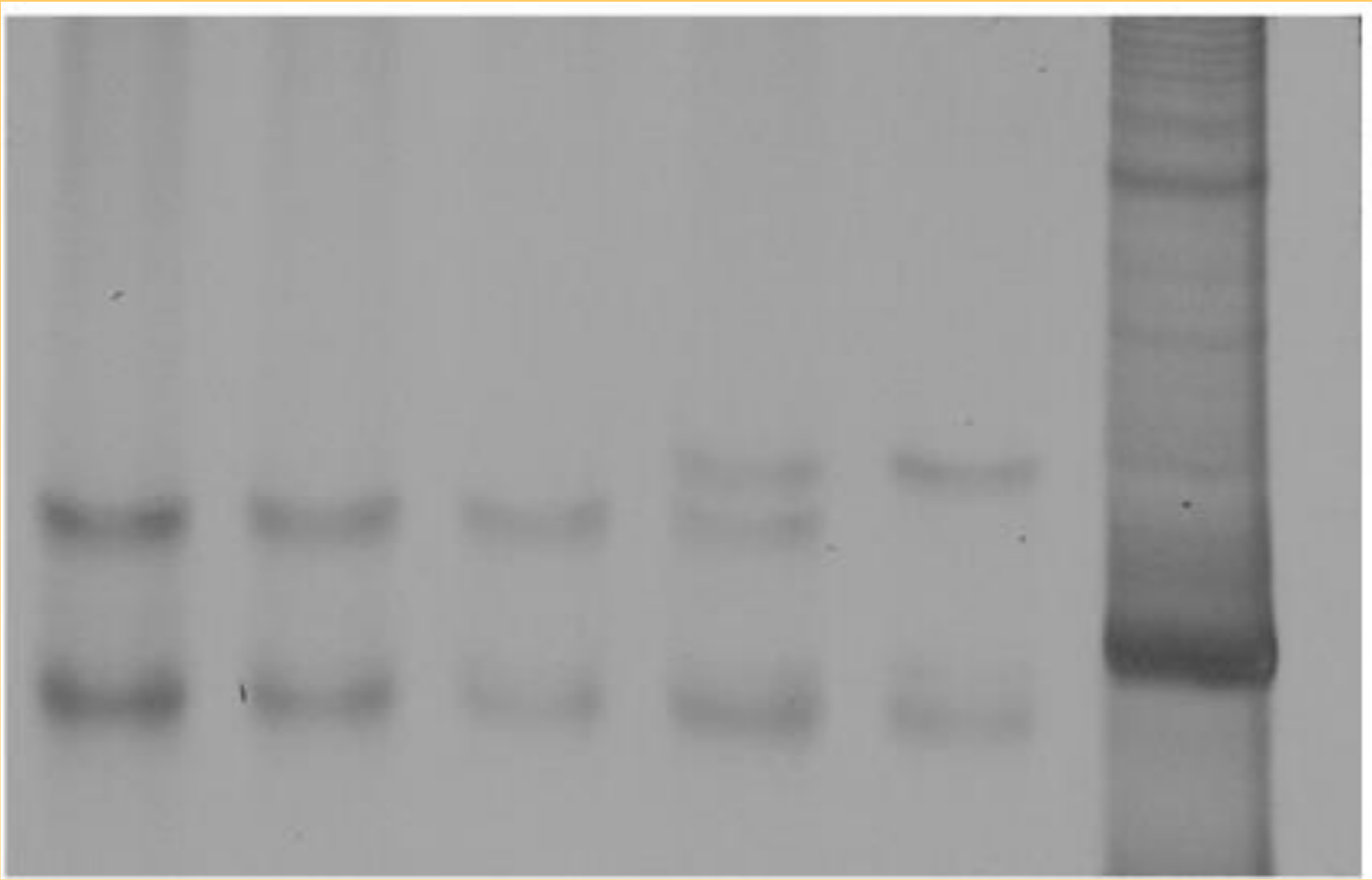
“Disease gene” Concept



In “simple disorders” we investigate one or few genes / proteins.

Looking for location (exon) harbouring mutation:
SSCP = single strand conformation polymorphism (exon amplification)





Typical electrophoresis gel showing SSCP analysis of a point mutation

From left to right: lanes 1-3 are homozygous for the wild-type, lane 4 is heterozygous for the mutant, lane 5 ishomozygous for the mutant and lane 6 is DNA marker.

GENOMICS

in cancer research

We, as most cancer researchers have long focused on mutations to a relatively small set of cancer-related genes as the decesive events in the transformation of healthy cells to malignant tumors.

Schematic representation of nucleotide changes found in Slovenian patients with gastric cancer

A92P

Found in 2 patients: 4 and 34.

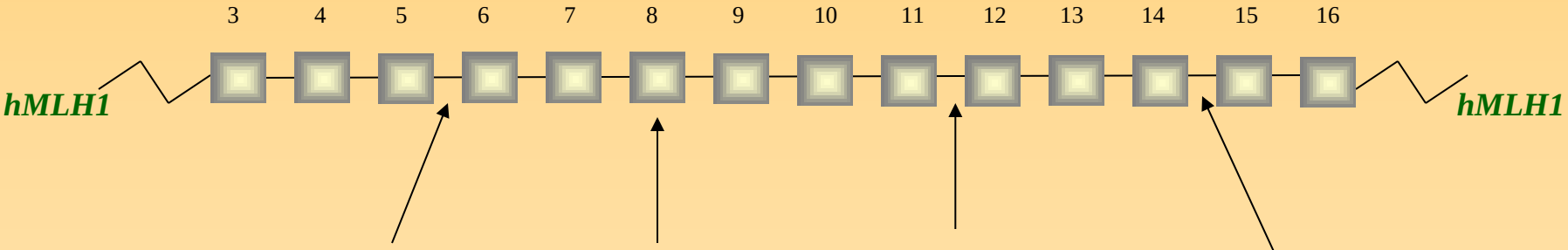
E433Q

Found in 1 patient: 34.

K618A

Found in 2 patients: 4 and 34.

Primer mutacijske analize konkretnega gena kot vpletenega v razvoj raka (GC).



IVS5 453+79 A>G

Found in 3 patients: 4, 38 and 63.

I219V

Found in 4 patients: 4, 34, 38 and 63.

1039-7 del $(T)_n$

Found in 5 patients: 34, 38, 56, 59 and 63.

1668-19 A>G

Found in 5 patients: 4, 34, 38, 56, and 63.

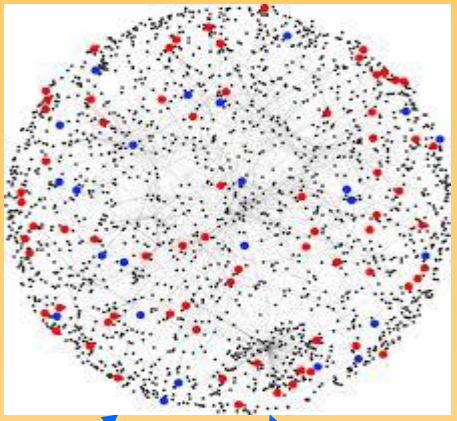
Shematic representation of nucleotide changes found in Slovenian patients with gastric cancer. Pathogenic mutations are in red, while polymorphisms are in black. Numbers above blocks are the numbers of exons of gene *hMLH1*.

Hudler P. et al., 2005

FUNCTIONAL GENOMICS

Post-genomic approach is based on functional genomics that considers a cell, at a physiological state under investigation, as a system of simultaneous, networking events at the levels of transcriptome and proteome.

Enonukleotidni polimorfizmi (SNP) posamično nimajo izrazitega fenotipskega učinka, v skupini pa so posledično odgovorni za razlike v kompleksnih proteinskih (npr. komunikacijskih) mrežah, ki se različno odzovejo na zunanjo motnjo, signal...



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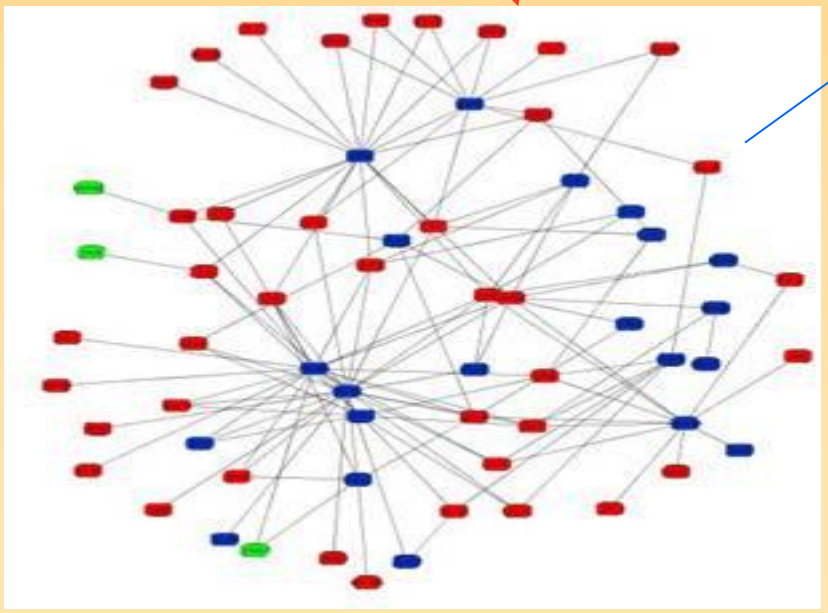
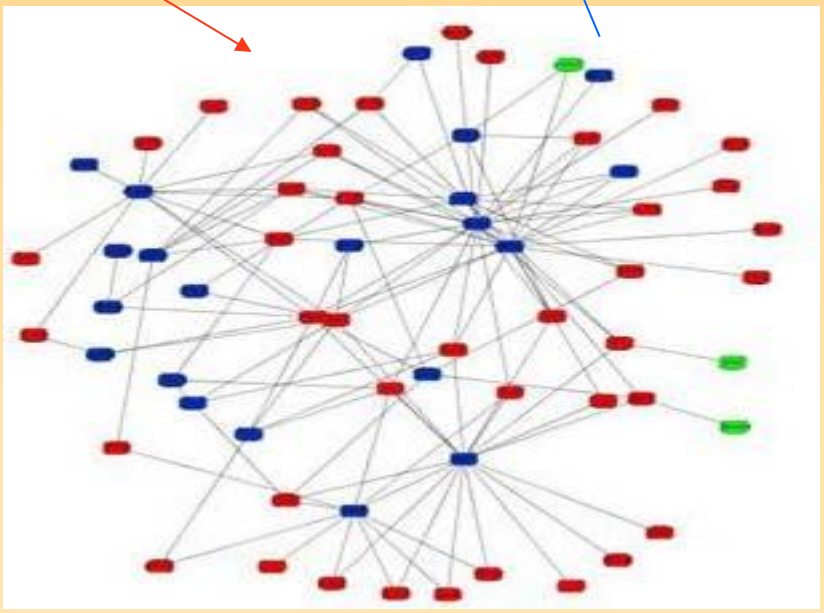
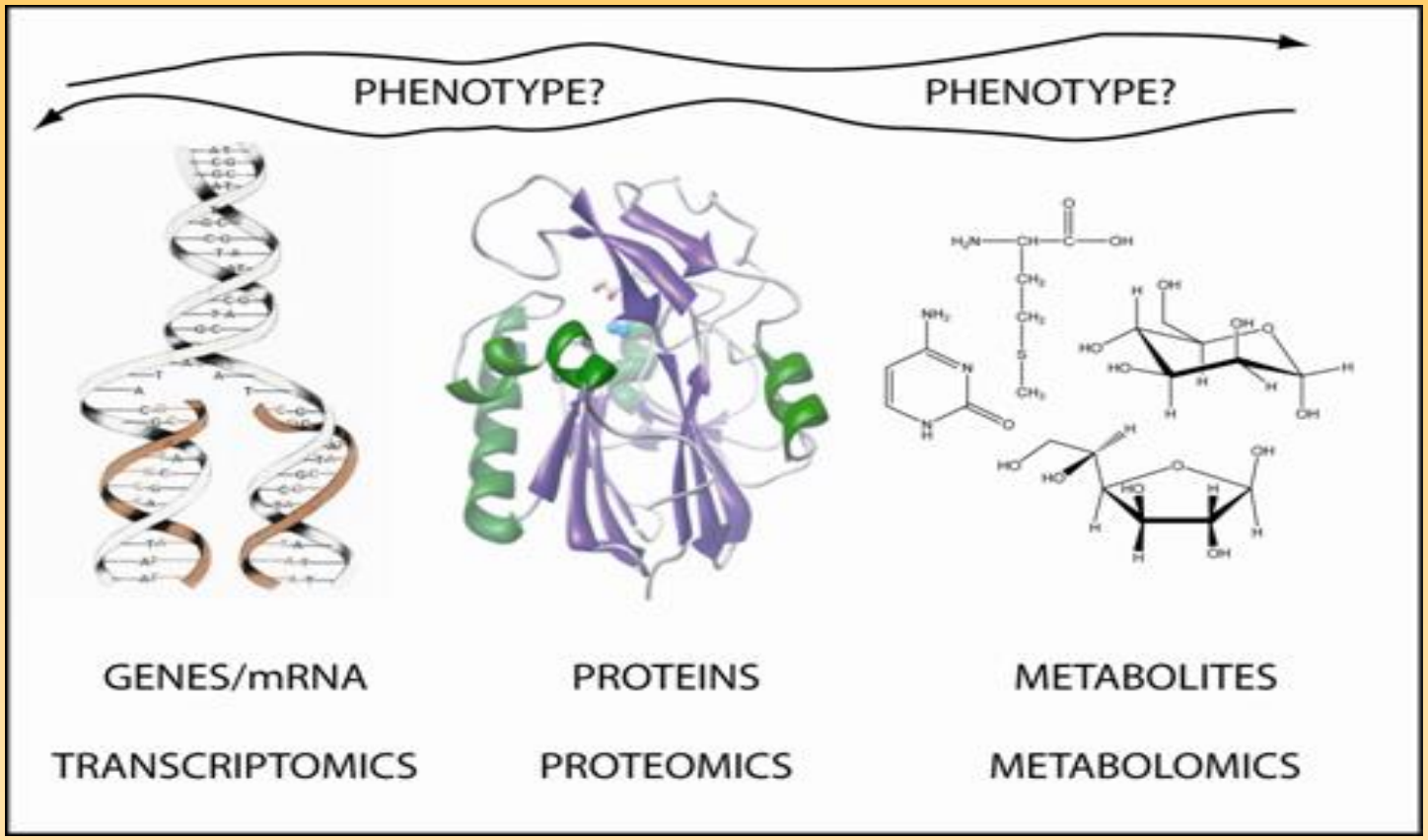


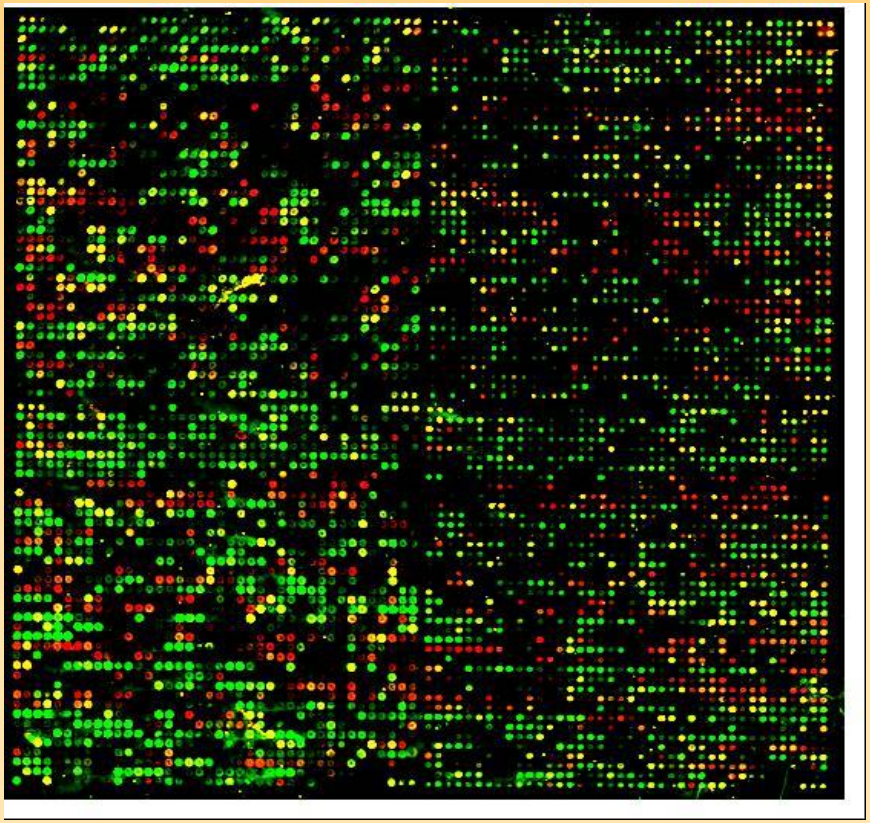




25,000-30,000	**several 100,000**	**up to 7,000**

FUNCTIONAL GENOMICS

Transcriptomics

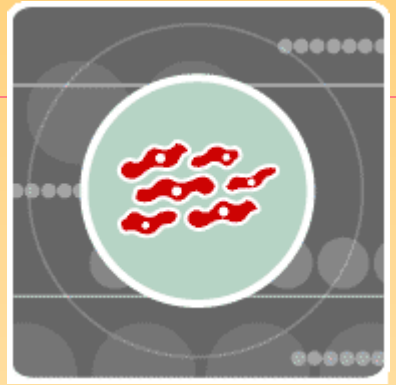


DNA Microarray Methodology Animation.htm

DNA Microarray Methodology Animation.htm

FUNCTIONAL GENOMICS

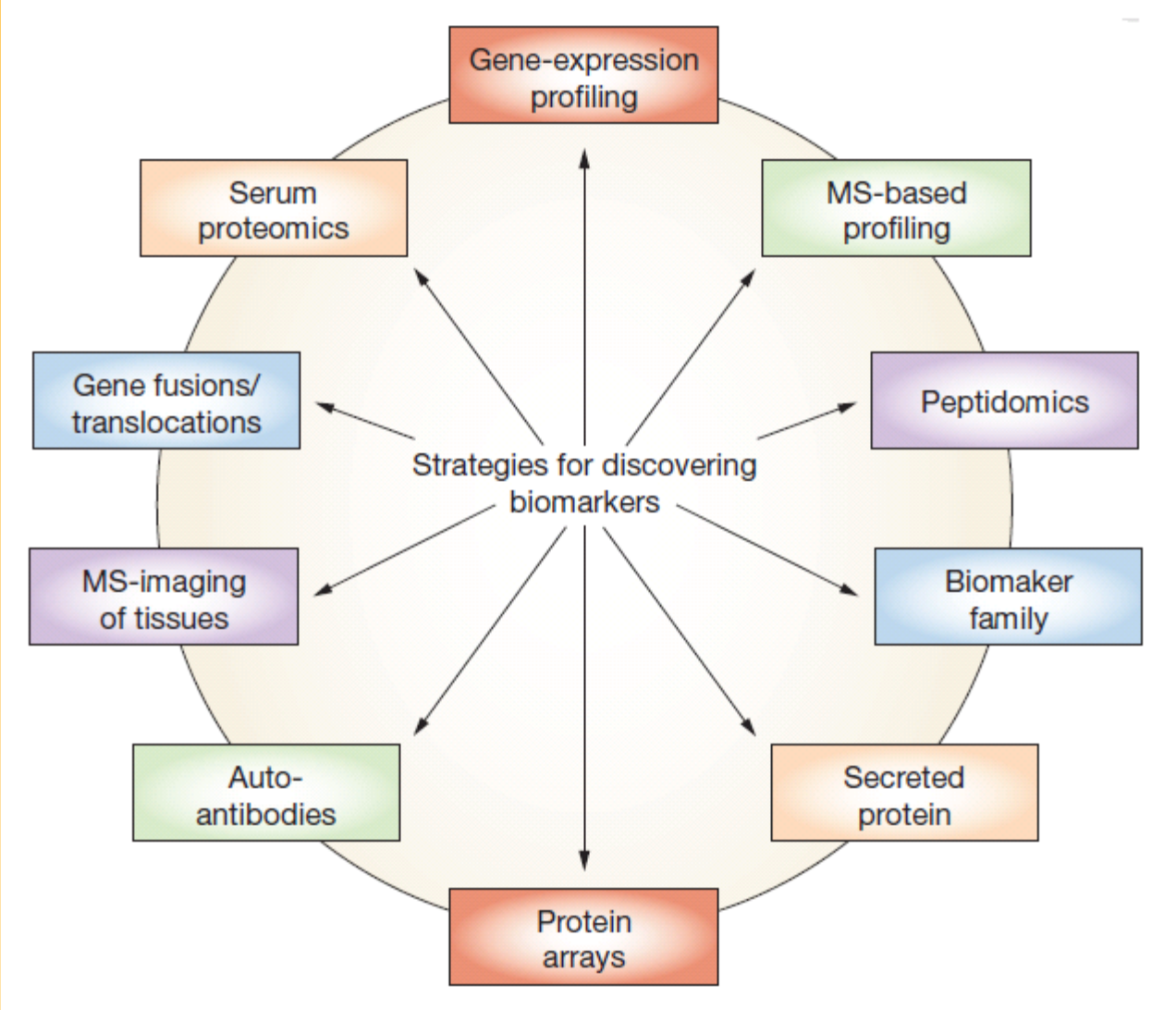
Proteomics



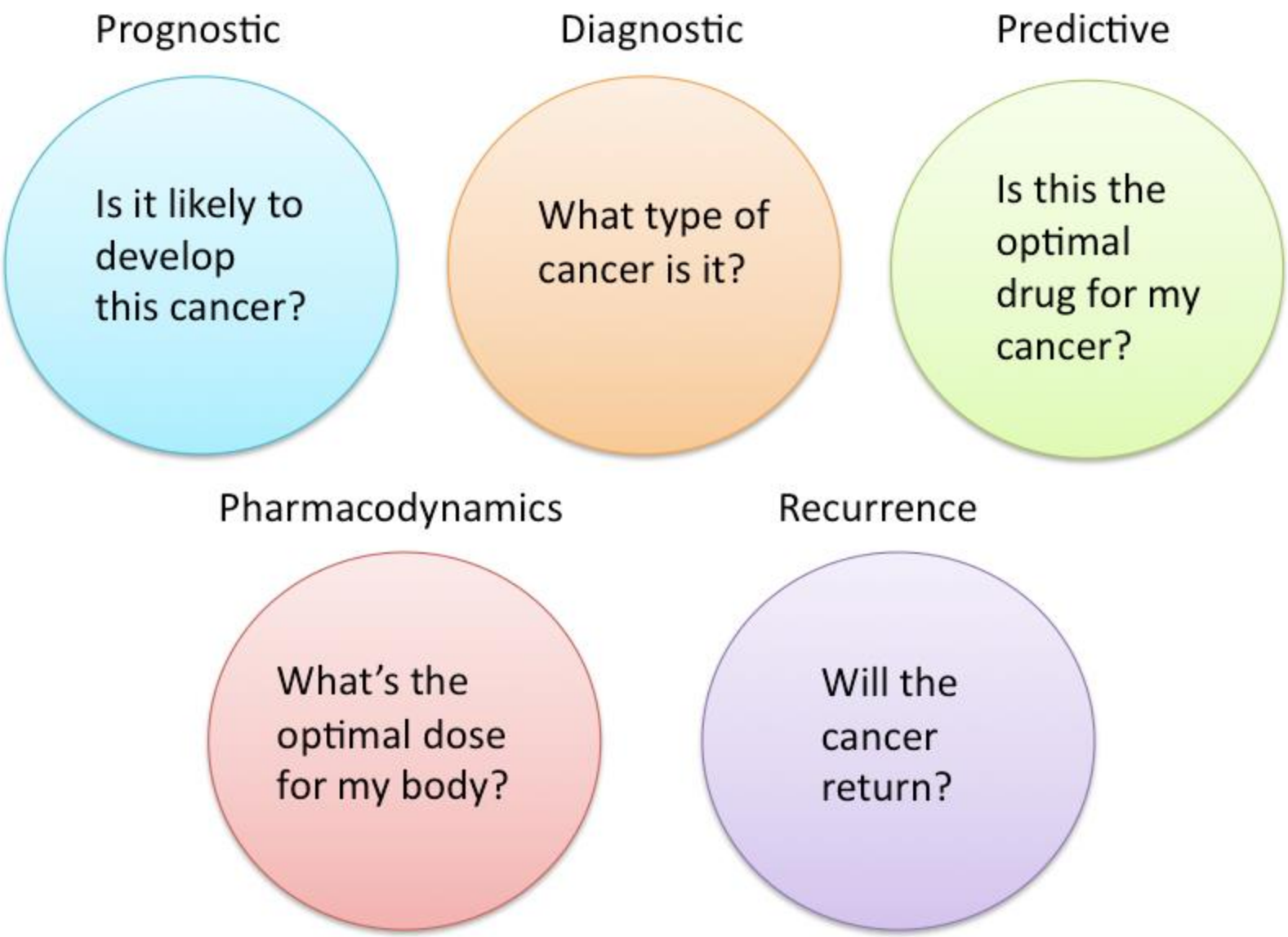
http://www.childrenshospital.org/cfapps/research/data_admin/Site602/mainpageS602P0.html

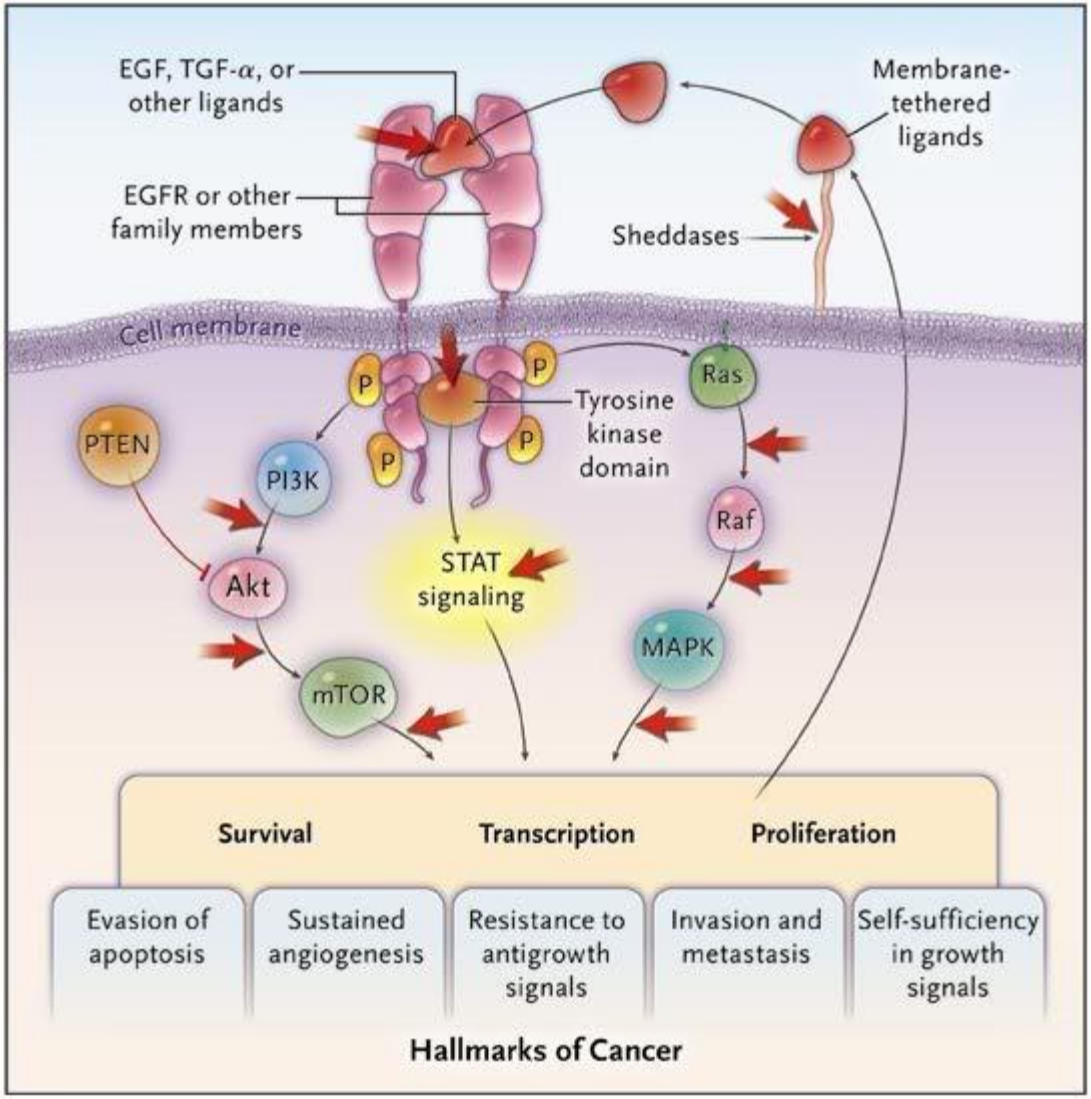
CANCER BIOMARKERS

in research & practice


Gene-expression profiling
Serum proteomics
MS-based profiling
Gene fusions/ translocations
Peptidomics
Strategies for discovering biomarkers
MS-imaging of tissues
Biomaker family
Auto-antibodies
Secreted protein
Protein arrays

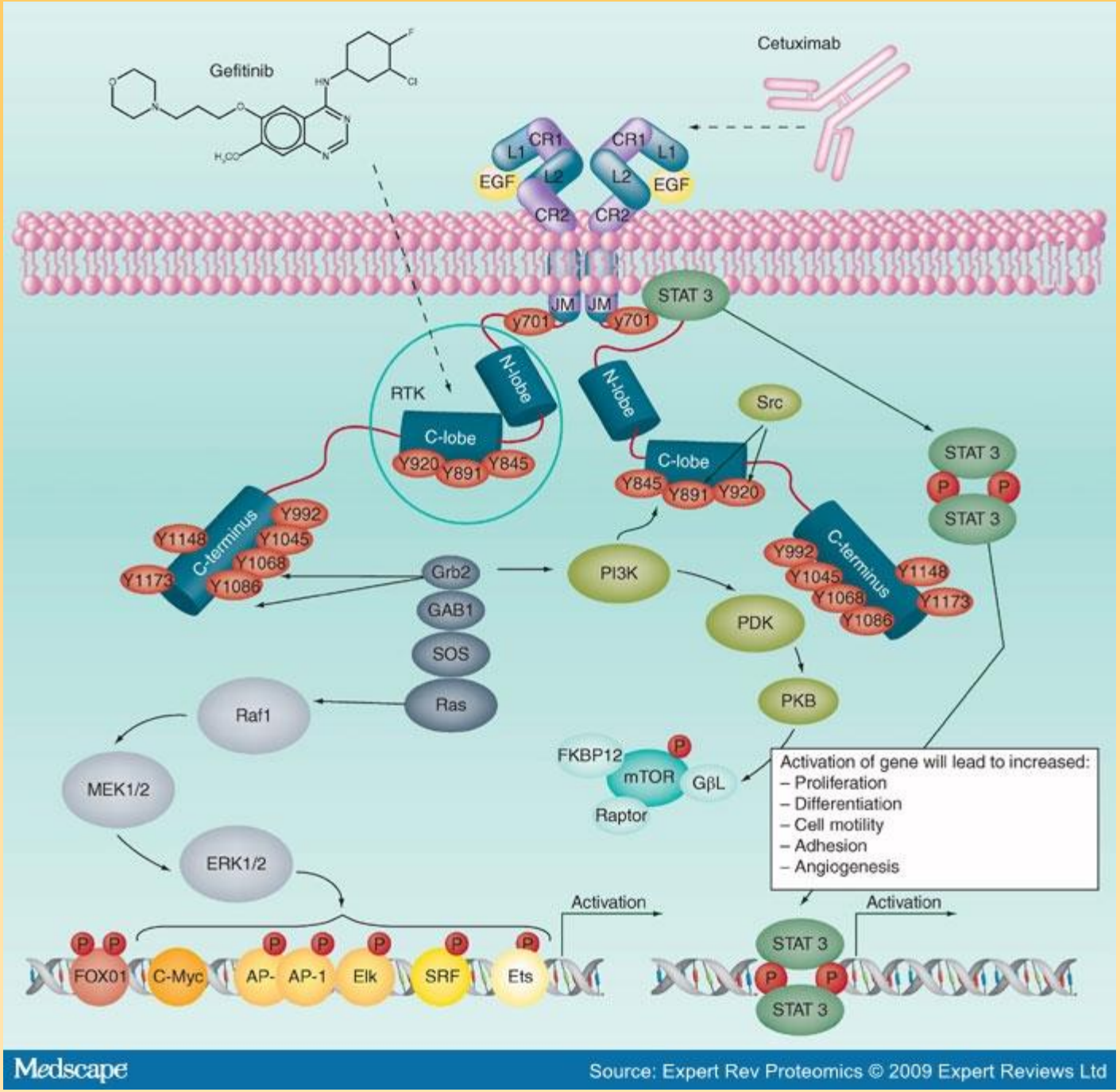
Questions that can be answered by cancer biomarkers



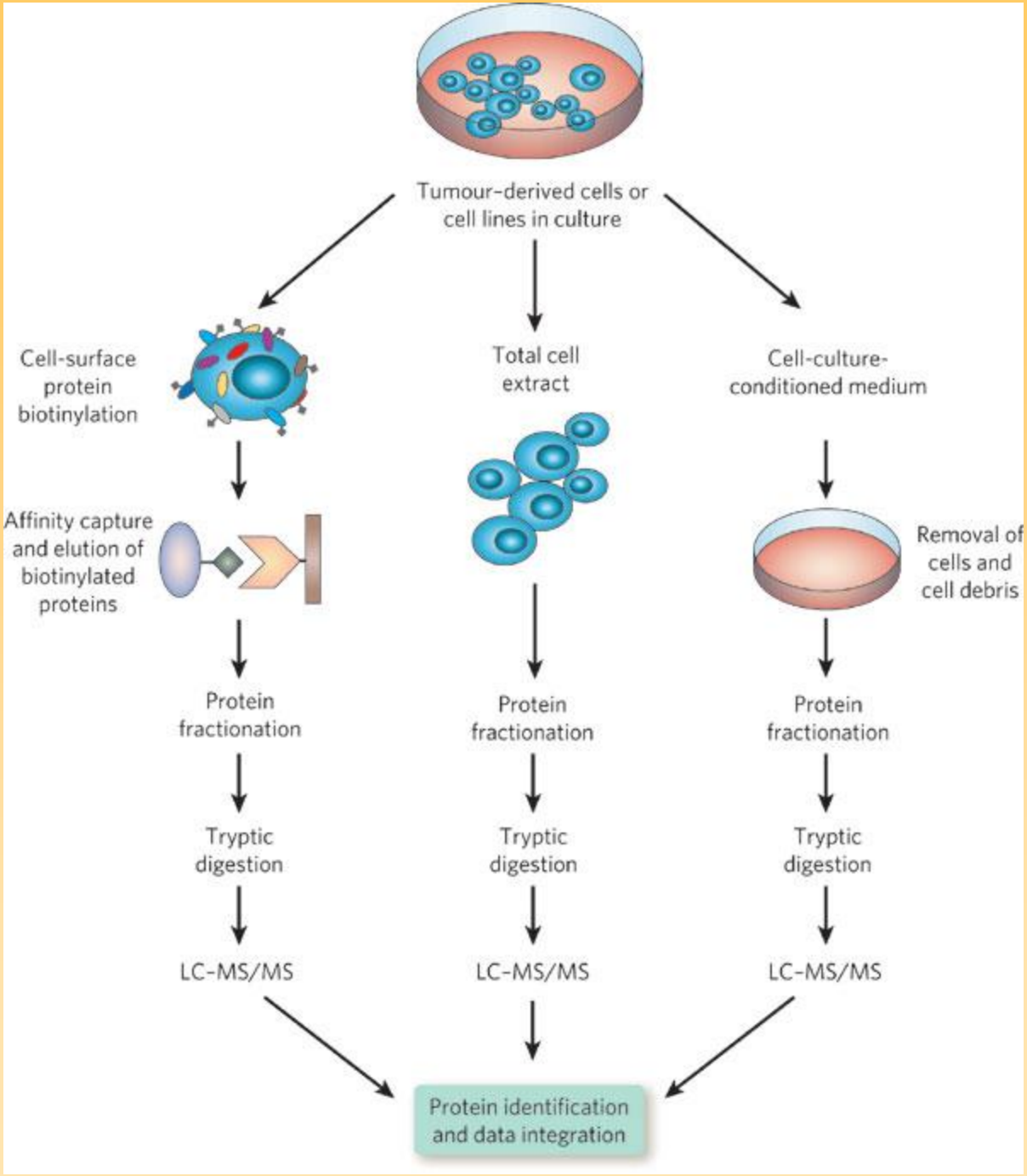

EGF, TGF-α, or other ligands
EGFR or other family members
Membrane-tethered ligands
Sheddases
Cell membrane
P
P
P
P
Tyrosine kinase domain
Ras
PTEN
PI3K
Raf
Akt
STAT signaling
MAPK
mTOR
Survival
Transcription
Proliferation
Evasion of apoptosis
Sustained angiogenesis
Resistance to antigrowth signals
Invasion and metastasis
Self-sufficiency in growth signals
Hallmarks of Cancer

Biooznačevalci kot tarče za zdravljenje raka:

Proti površinskim proteinskim biooznačevalcem običajno razvijamo blokirajoča monoklonska protitelesa, proti notranjim pa specifične male molekule (preko računalniškega, virtualnega „dockinga“).



Običajni pristopi proteomike.



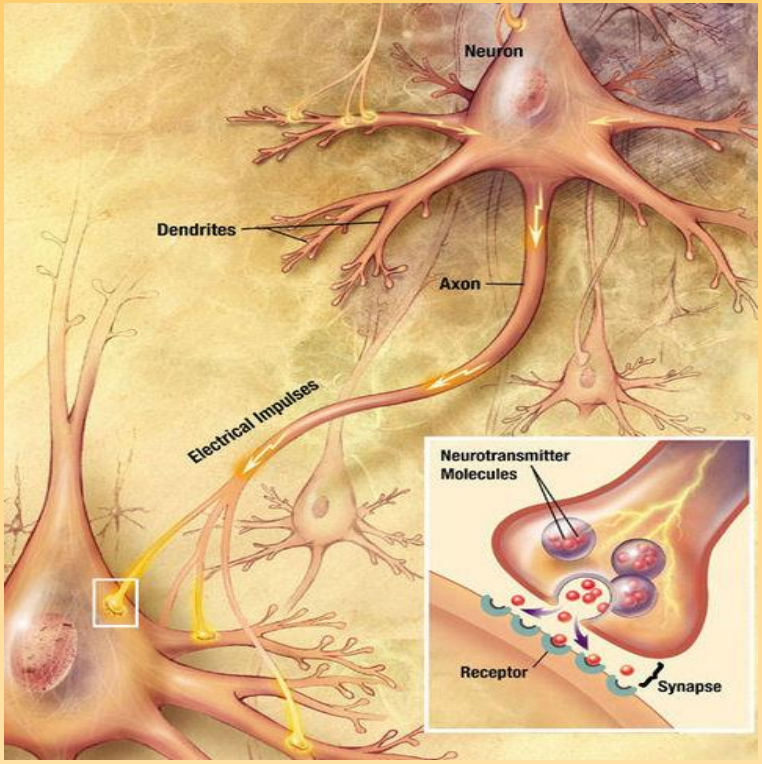
Seznam ugotovljenih označevalcev matičnih celic raka, ki pa na žalost niso zelo specifični, saj jih izražajo tudi številne celice zdravih tkiv kot tudi normalne matične celice.

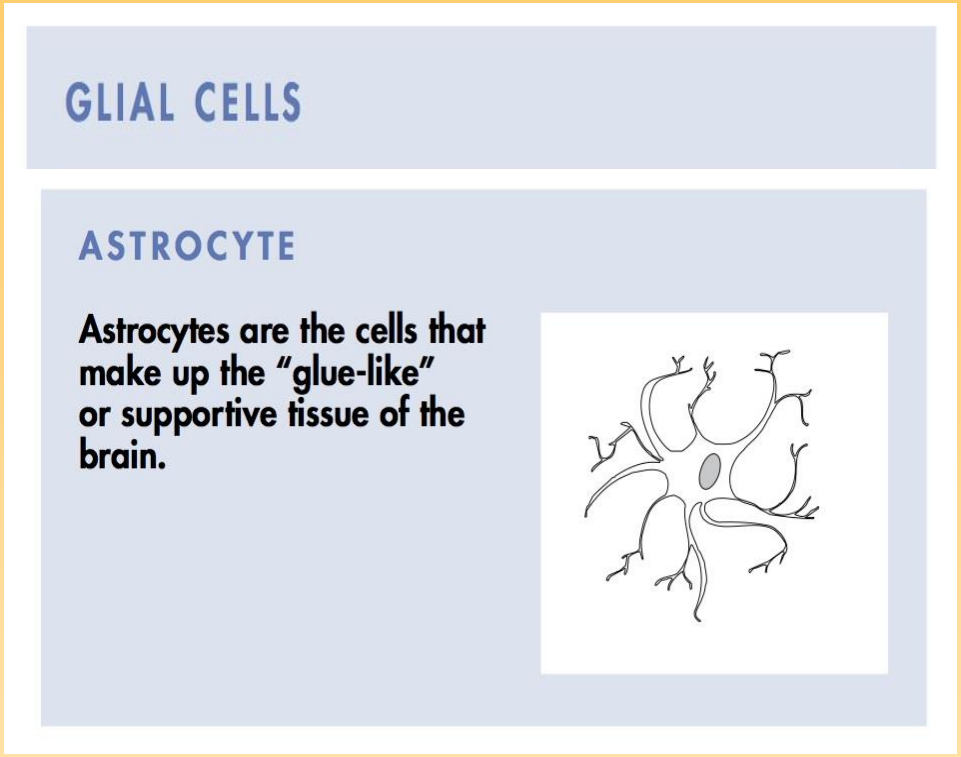
Table 1: Surface markers used for the identification of CSCs

Marker	Expression in healthy tissue	Marks cancer stem cells in
ABCB5,ATP-binding cassette transporter B5		
EpCAM, epithelial cell adhesion molecule		
ESA,	epithelial-specific antigen.	
CD19	Broadly on B lymphocytes	B cell malignancies
CD20	Broadly on B lymphocytes	Melanoma
CD24	Broadly on B cells; neuroblasts	Pancreas/lung cancer, negative on breast cancer
CD34	Hematopoietic and endothelial progenitors	Hematopoietic malignancies
CD38	Multiple stages of B and T cells	Negative on AML
CD44	Broadly on many tissues	Breast/liver/head and neck/pancreas cancer
CD90	T cells, neurons	Liver cancer
CD133	Proliferative cells in multiple organs	Brain/colorectal/lung/liver cancer
EpCAM/ESA	Panepithelial marker	Colorectal cancer, pancreatic cancer
ABCB5	Keratinocyte progenitors	Melanoma

Nadaljujem s primerom iskanja proteomskih označevalcev karcinogeneze pri glioblastomu.

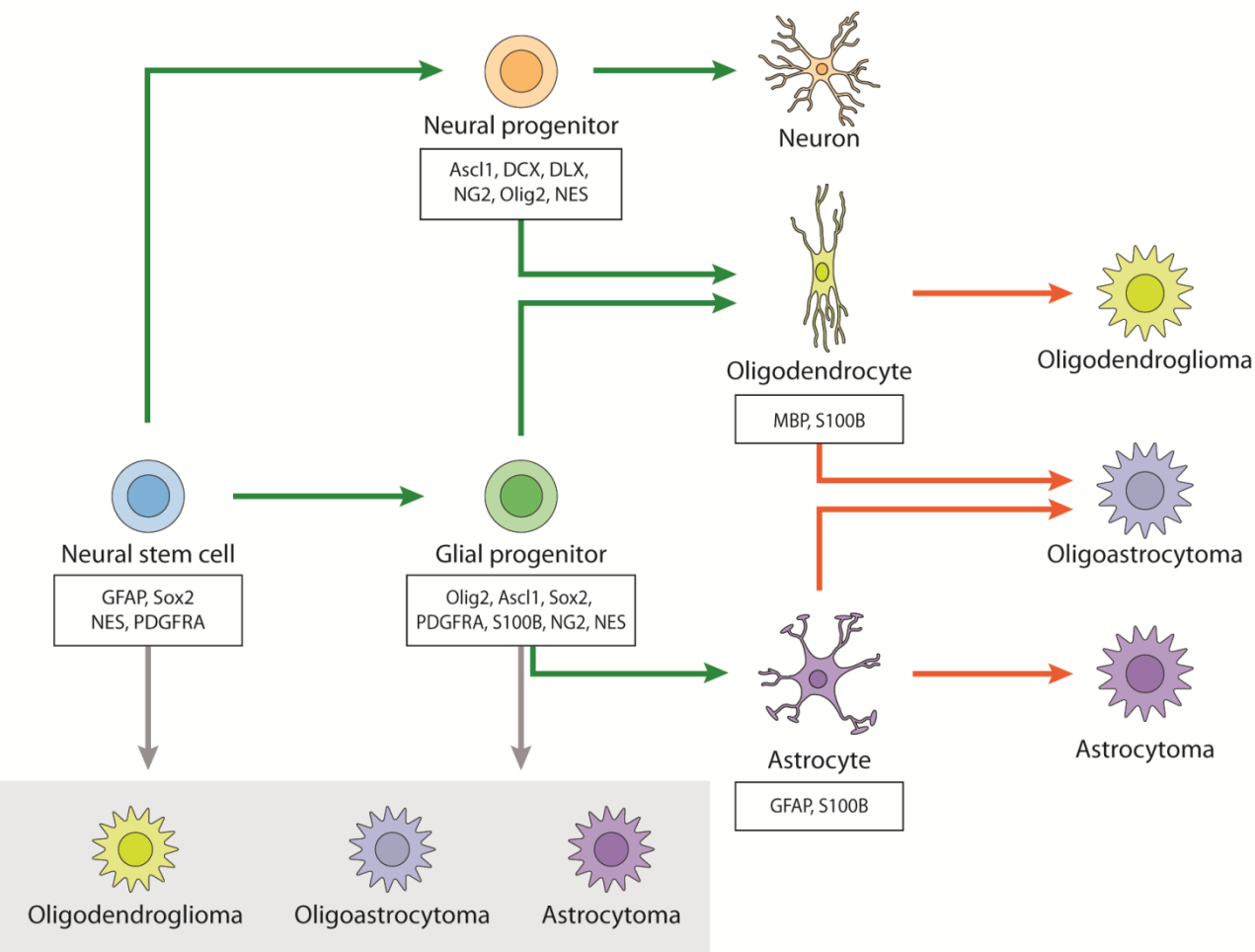
"Glioma" is a general term used to describe any tumor that arises from the supportive ("gluey") tissue of the brain. This tissue, called "glia," helps to keep the neurons in place and functioning well.





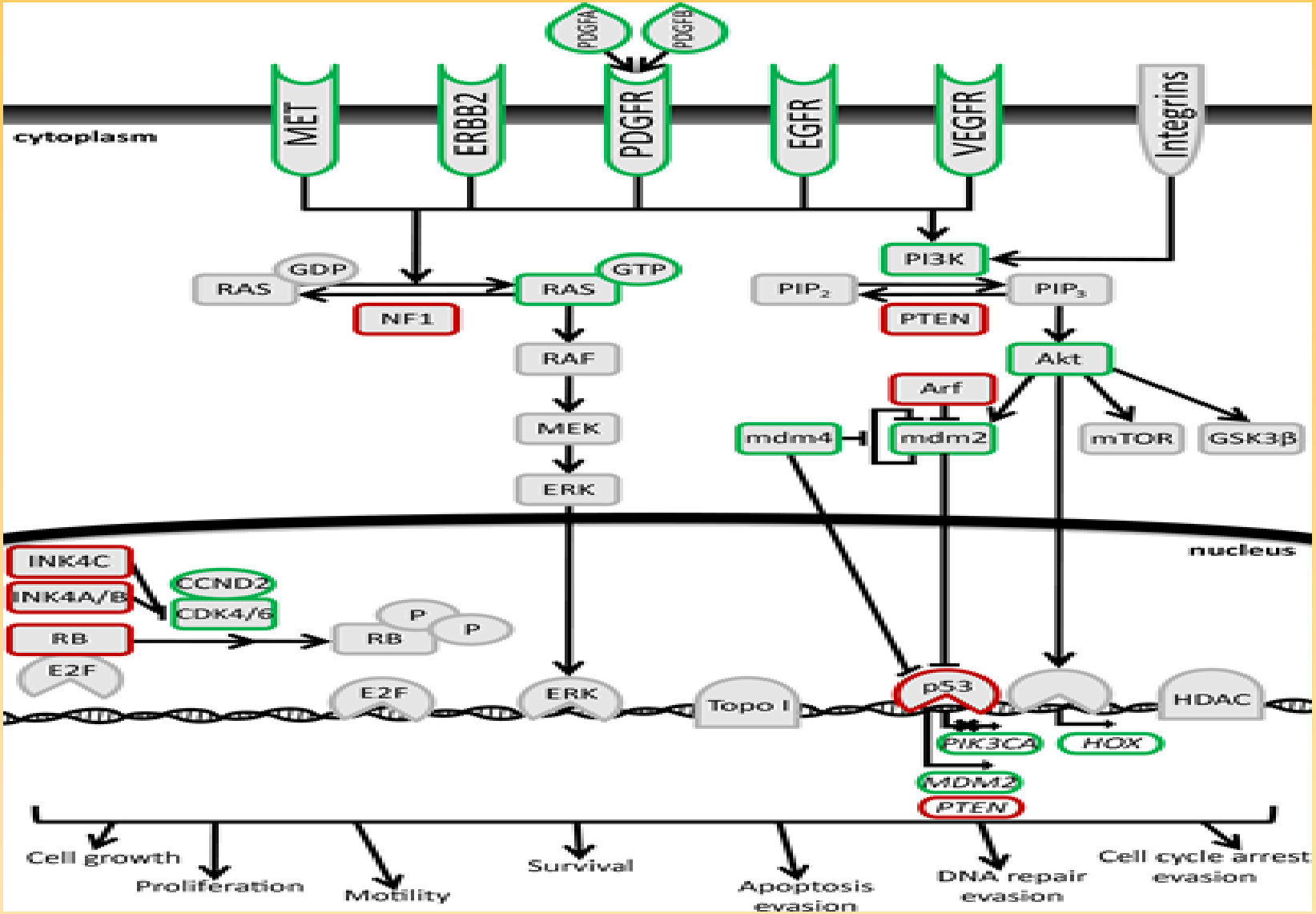
There are three types of normal glial cells that can produce tumors. An astrocyte will produce astrocytomas (including glioblastomas), an oligodendrocyte will produce oligodendrogliomas, and ependymomas come from ependymal cells. Tumors that display a mixture of these different cells are called mixed gliomas.

www.ABTA.org, 2013



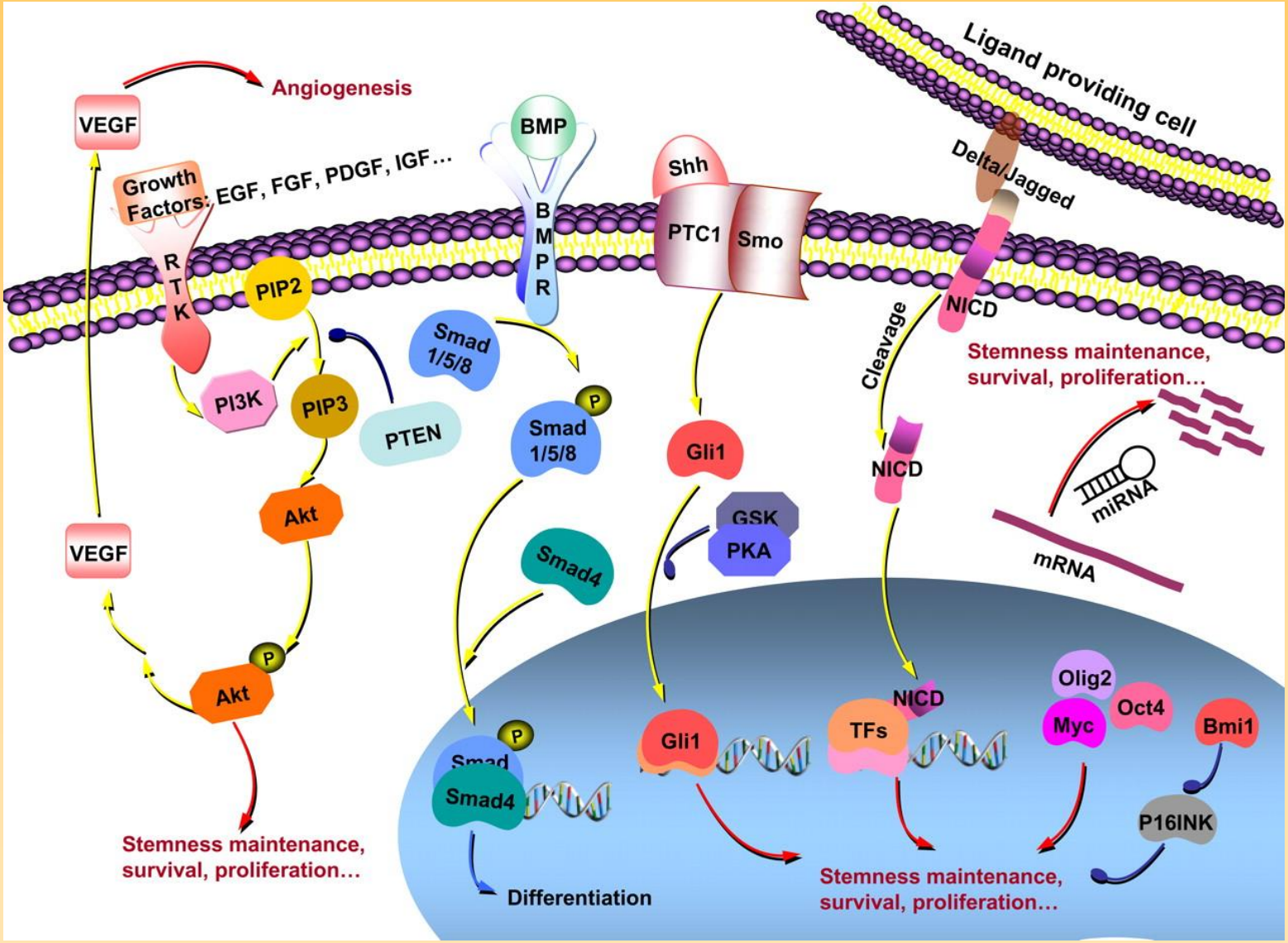
The normal differentiation process originates three main types of cells in the mature CNS: neurons, glial cells (oligodendrocytes, astrocytes, ...), and ependymal cells (not shown). Malignant transformation occurs through didefferentiation process either from glial cells or from glia progenitor cells and/or from neuronal stem cells.

Celine S. et al., *INTECH, 2013*



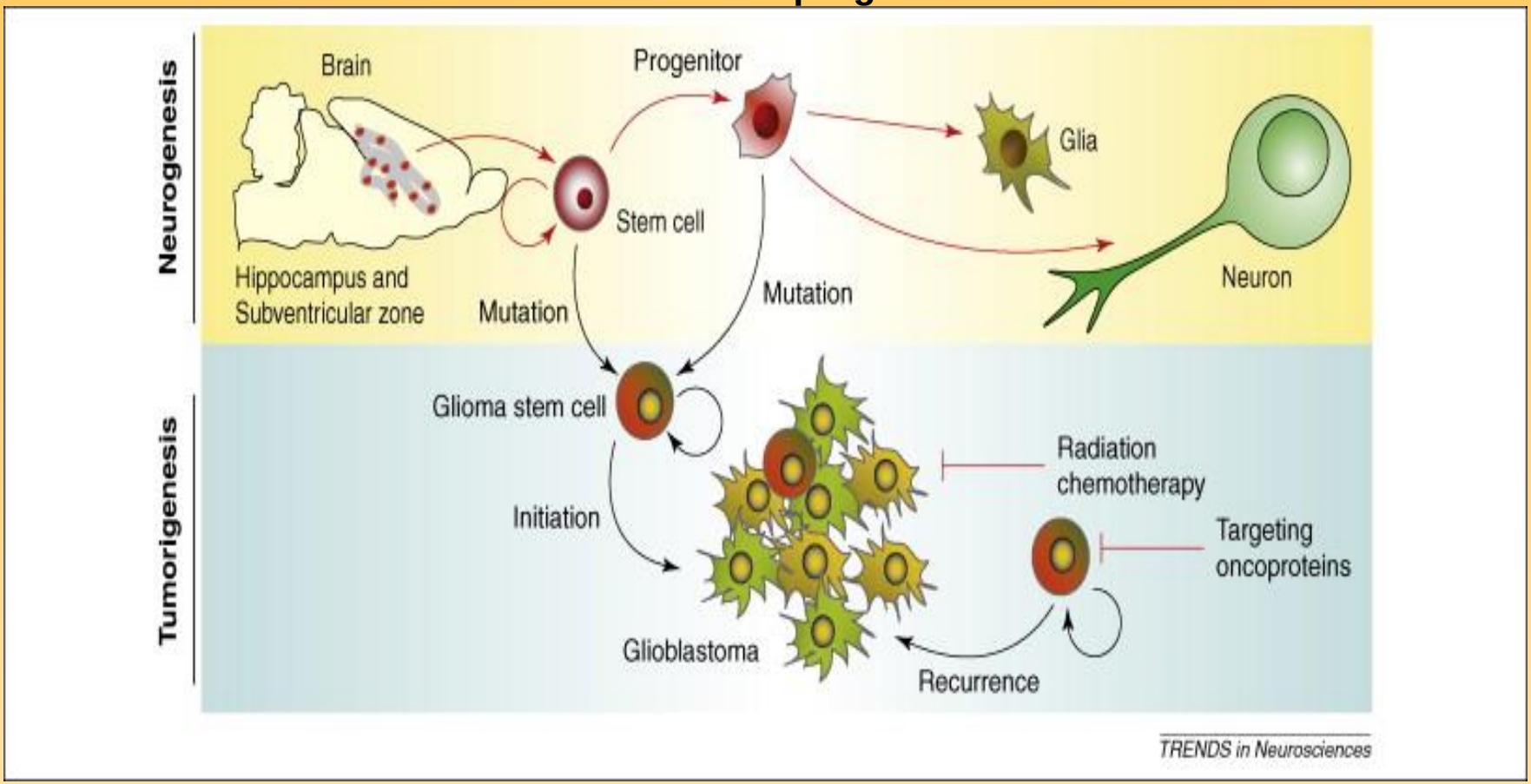
Common genetic alterations in GBM affect the RB, p53 and RTKs pathways: activation of oncogenes and loss / inactivation of suppressor genes.

Complex signaling pathways and cellular factors regulate glioma CSCs. Glioma CSCs are controlled at multiple levels by complicated regulatory networks.



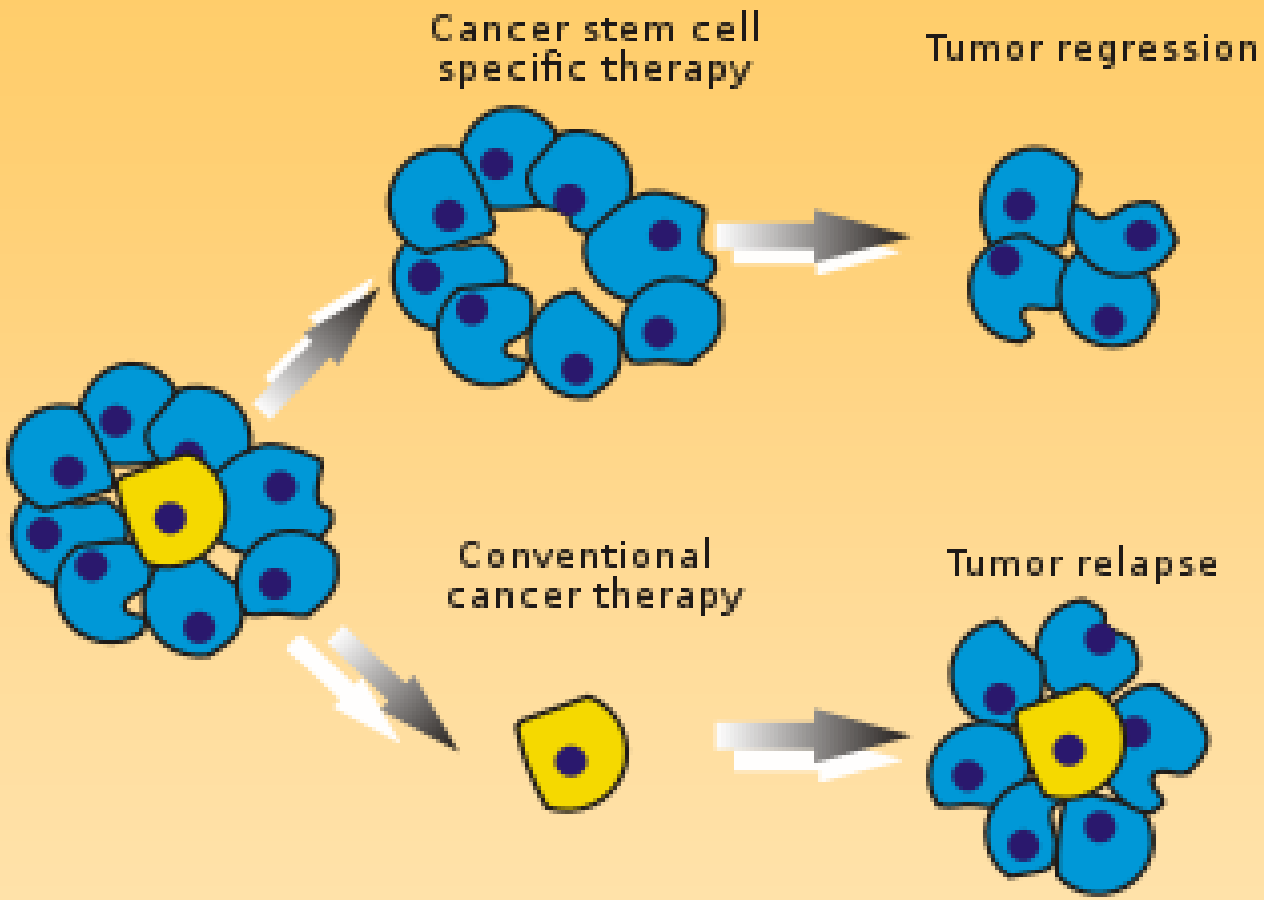
Li Z et al. J. Biol. Chem. 2009;284:16705-16709

Characteristics and fates of normal neural progenitor cells and cancer stem cells.



Cancer stem cells within a brain tumor can arise from normal stem cells or from neural progenitor cells that harbor mutations. Cancer stem cells within glioblastomas can be particularly resistant to cytotoxic therapies, and therefore are a source of cells for cancer recurrence. To meet the challenge of a cure for glioblastoma, new therapies that selectively suppress proliferation and/or kill cancer stem cells must be found.

Zhang P. et al, *TIN, 2009*



From Wikipedia, 2013

Biomarkers:	Can be measured in:
-**diagnostic**	**- cerebrospinal fluid (CSF)**
- **prognostic**	**- serum**
- **predictive**	**- tumor tissue**
- **pharmakodynamic**	

Glioblastoma

Immunohistochemistry: MGMT protein, EGFRvIII, IDH1 R132s variant

CSF: There is no diagnostic, prognostic or predictive CSF marker in clinical use. [bFGF, VEGF; MIC-1/GDF15, deoxytimidine kinase, NSE, tenascin neuronal stem cell marker prominin-1/CD133; GELSOLIN, Rho GDP-dissociation inhibitor α, IGHG1...]

Serum: EGF, glycoprotein YKL-40; cathepsin D, caldesmon 1-CaD, recoverin, GFAP

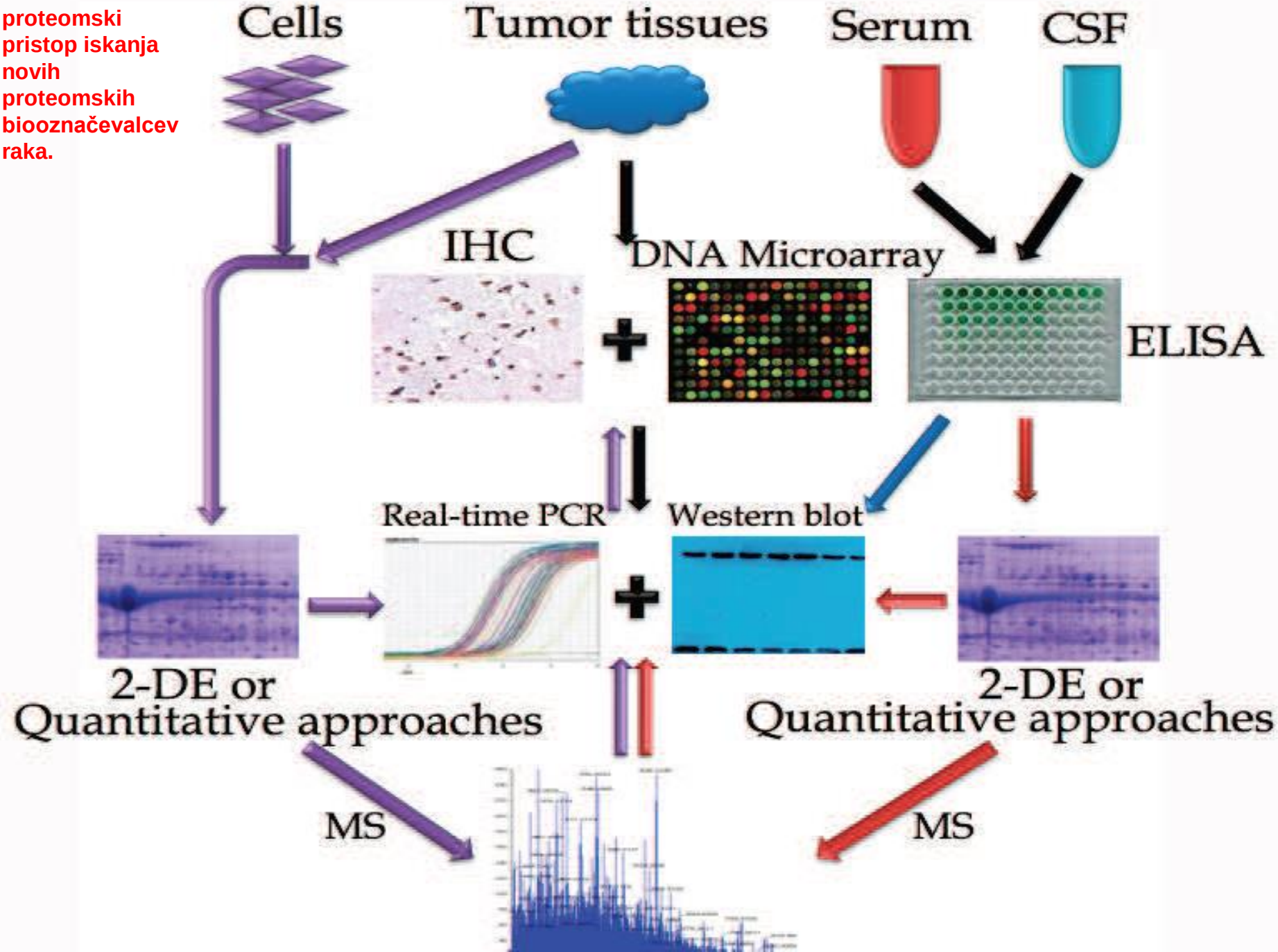
Molecular and genetic markers: MGMT, IDH1 / IDH2, Δ 1p & 19q

Predictive for drug response: EGFRvII, PTEN

Stem cell markers: CD133, A2B5, CD15; podoplanin, integrin alpha 6

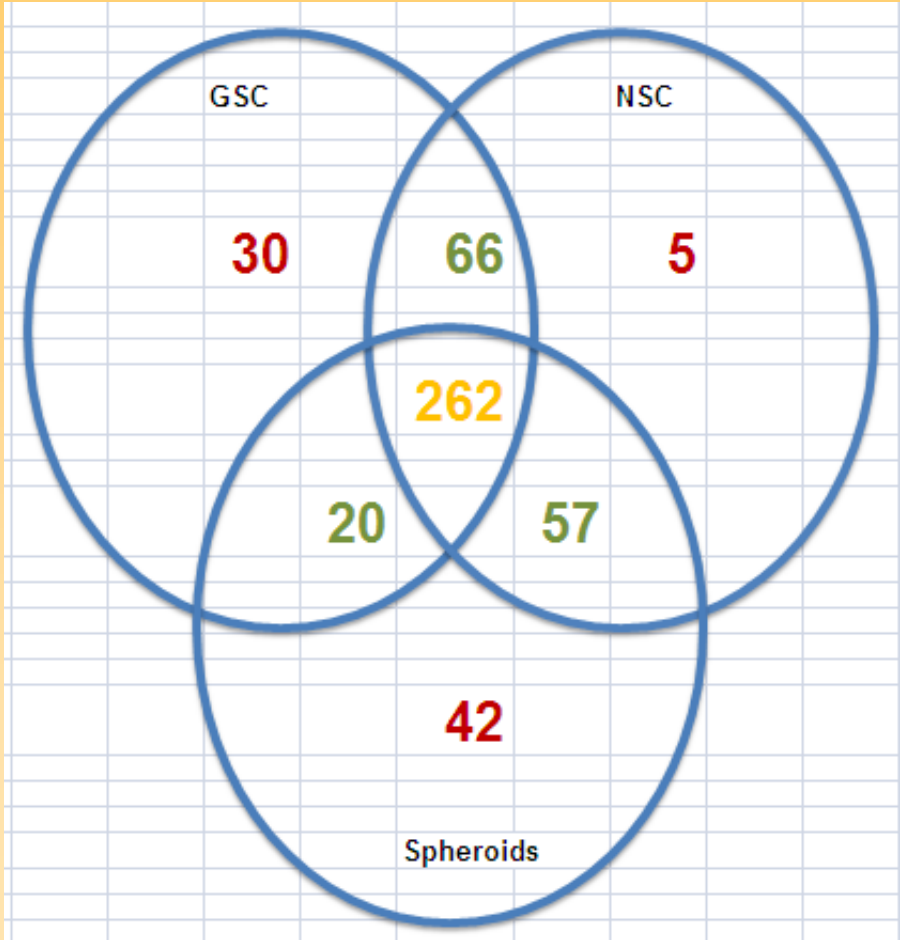
Ugotovitev: pomanjkanje specifičnosti biooznačevalcev samo za matične celice raka (glioblastoma).

Konvencionalen proteomski pristop iskanja novih proteomskih biooznačevalcev raka.



Database of differential proteins

- Venn diagram with data after BH correction of p-values, from the “3-way comparison“
  - 1982 proteins detected;
  - 727 proteins differentially expressed (with $p < 0.05$) among the three groups;
  - 482 proteins differentially expressed (with $pBH < 0.05$ – corrected p-value).



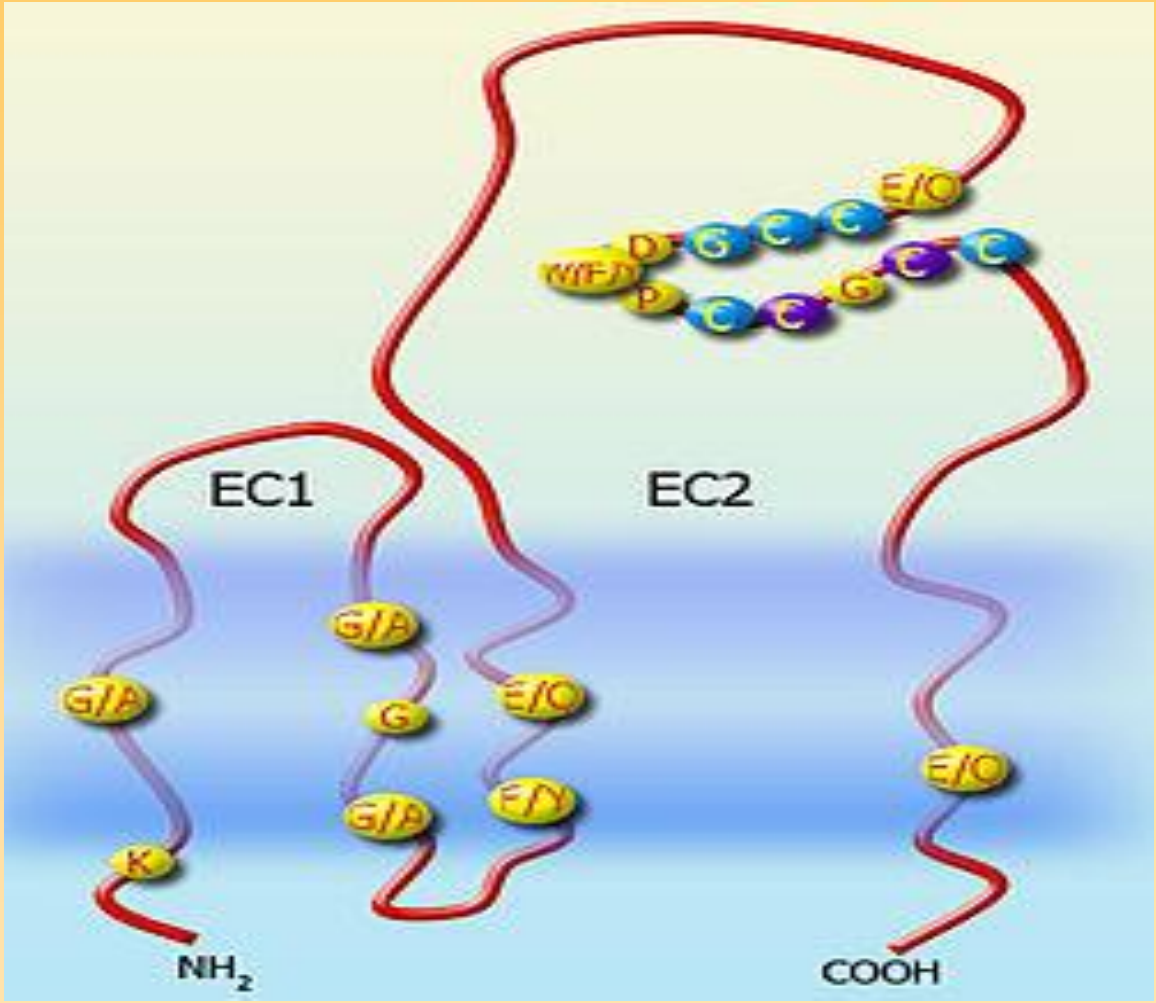
U.Rajčević, T.Lah Turnšek et al., GLIOMA, 2011.

Proteins specific to GSC ar common to GSC & GBM spheroids

Cells	EGFR	FGFR	EGFR & FGFR
GSC (NCH cell lines)	KRR1 (RIP-1)	EBNA1BP2	CDKN2A, CDK4
GSC & GBM spheroids	HIBADH	YARS, COL11A1, SERPINE1 (PEDF)	CD9, DNM-2

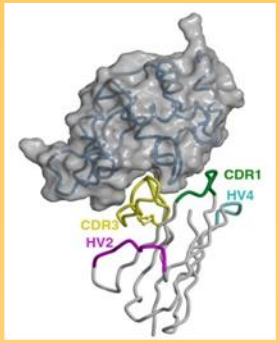
Selection of 4 candidates (FDR<0.05)

Protein	Protein name	FDR value	Specificity
KRR1 (RIP-1)	Minor processome component	0.036185	GSC (NCH)
DNM-2	Dynamin 2 component	0.048839	GSC & GBM spheroids
SERPINE1 (PEDF)	Serpine peptidase inhibitor isophorm 1	0.047688	GSC & GBM spheroids
CD9	Antigen CD9	0.002746	GSC & GBM spheroids



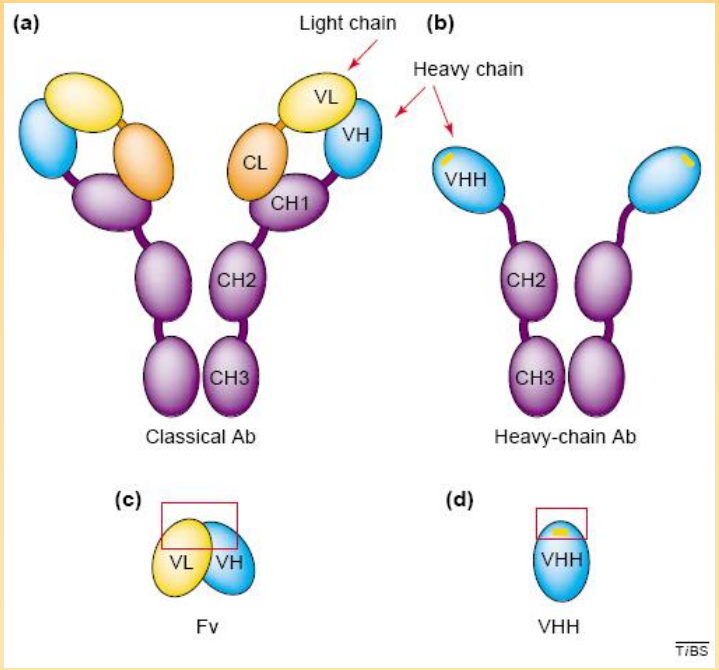
Tetraspanin protein family; CD9 antigen

http://en.wikipedia.org/wiki/Tetraspanin



Nano-protitelesa kot orodje za nov pristop iskanja proteomskih označevalcev raka.

CAMELIDAE (camels, llamas) heavy chain ANTIBODIES



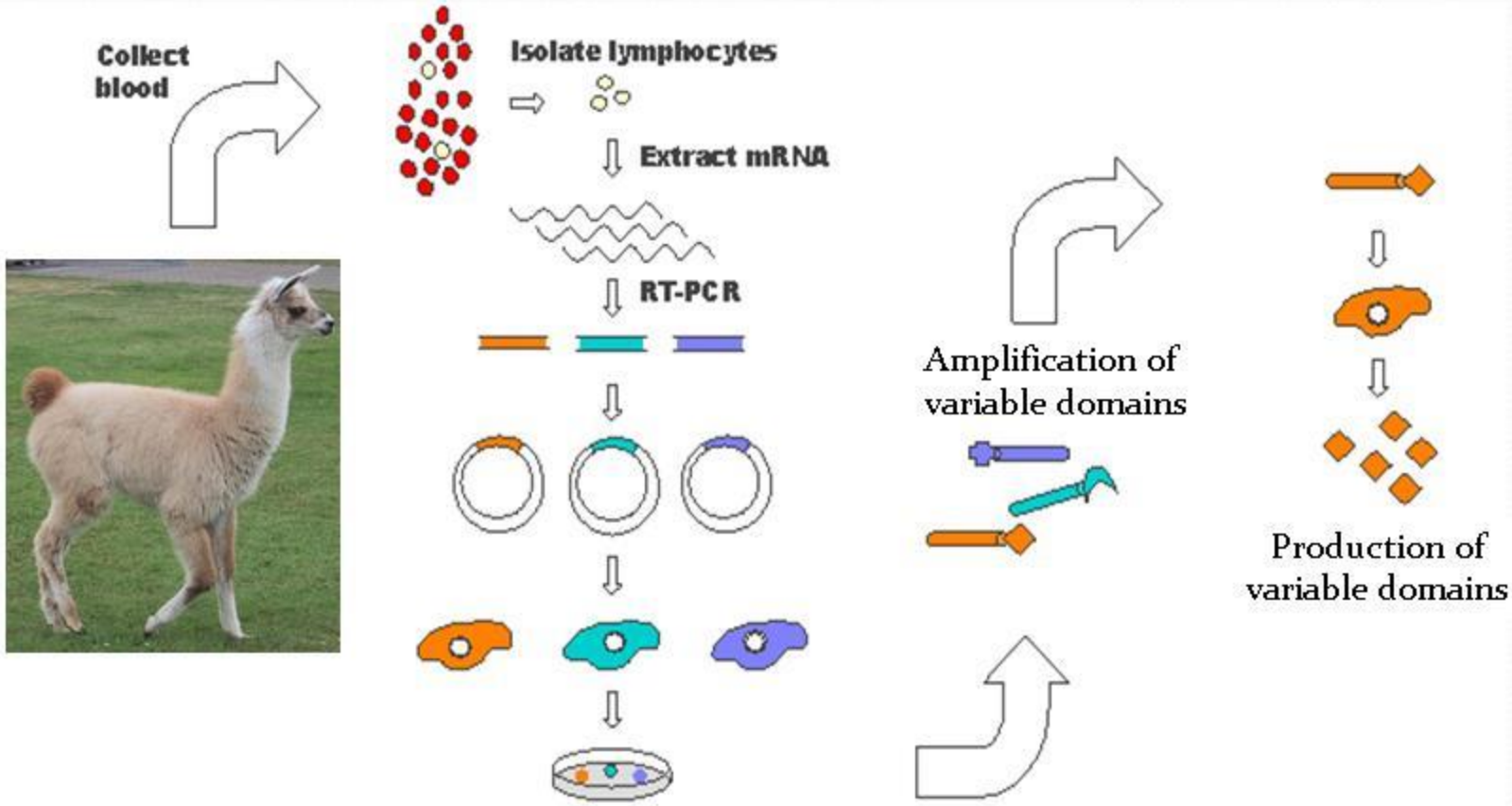
50 %

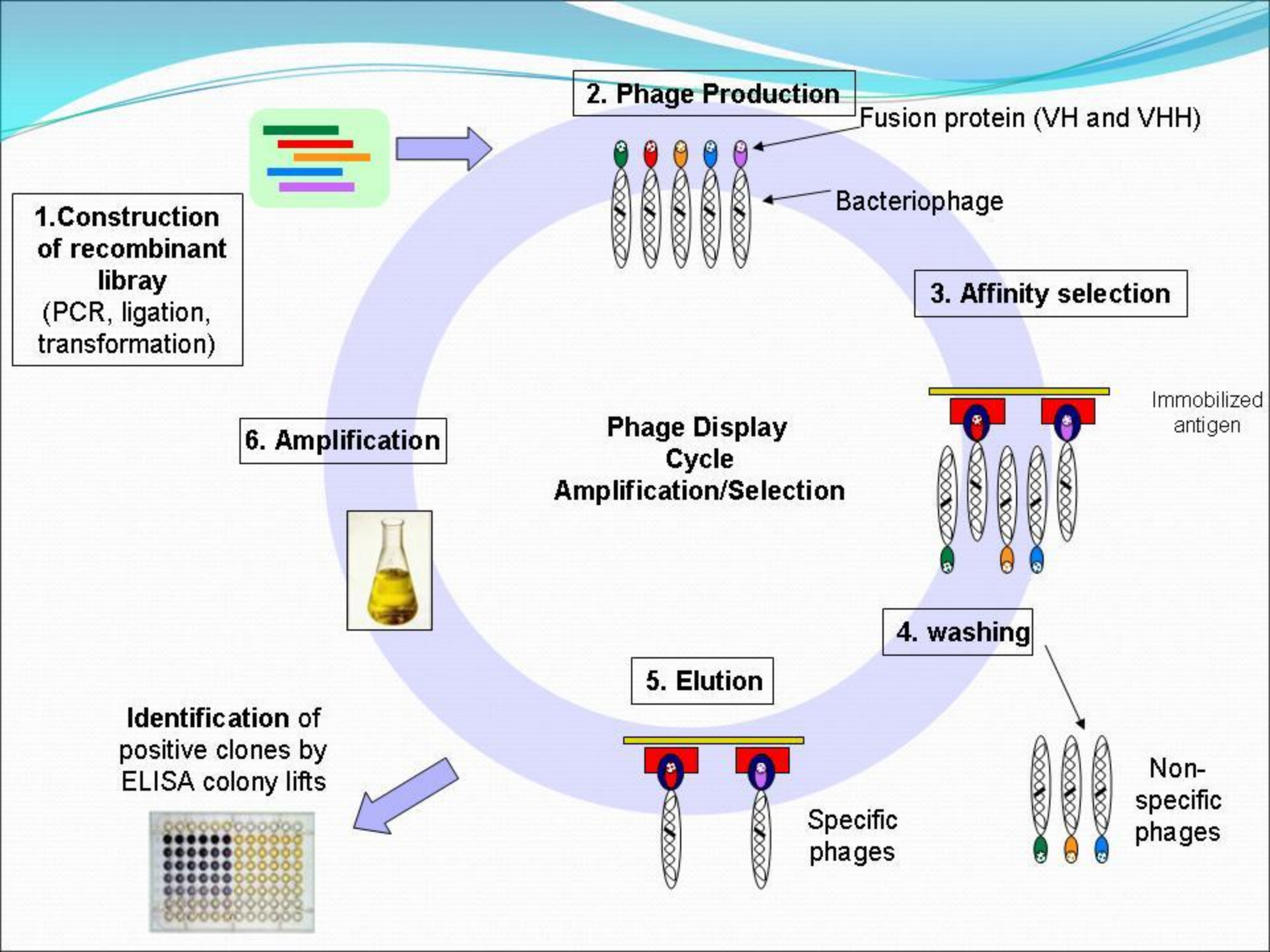
50 %

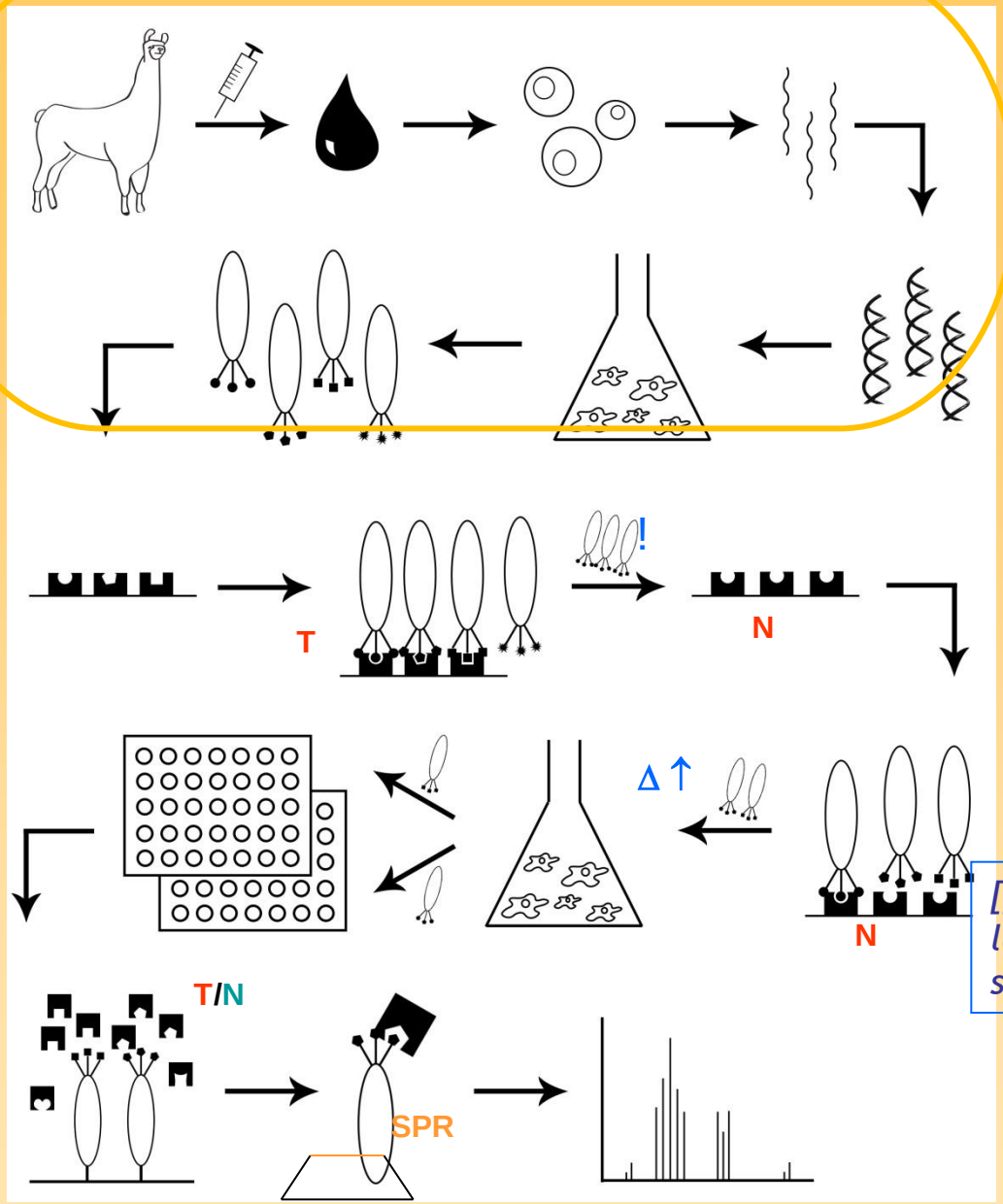
VHH:

- single domain, small size (15 kDa)
- high expression levels
- soluble and stable
- easy to handle compared to scFv

A. RECOMBINANT ANTIBODY LIBRARY CONSTRUCTION




2. Phage Production
Fusion protein (VH and VHH)
Bacteriophage
1.Construction of recombinant libray (PCR, ligation, transformation)
3. Affinity selection
Immobilized antigen
Phage Display Cycle Amplification/Selection
6. Amplification
4. washing
5. Elution
Identification of positive clones by ELISA colony lifts
Specific phages
Non-specific phages



immunization

blood removal

lymphocytes

RNA extraction

cDNA synthesis

PCR and E. coli cloning

phage display

panning

[protein extract from] laminin-grown GBM stem enriched cells

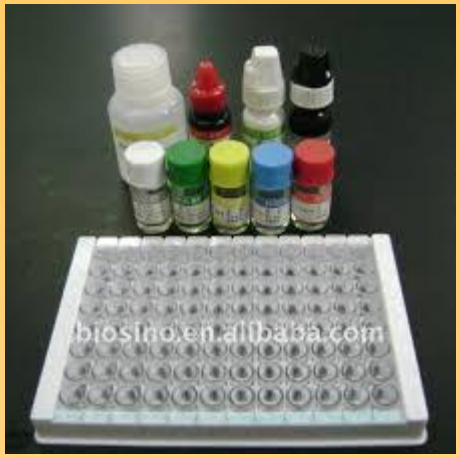
GBM tissue

differential screening

vs. normal brain tissue

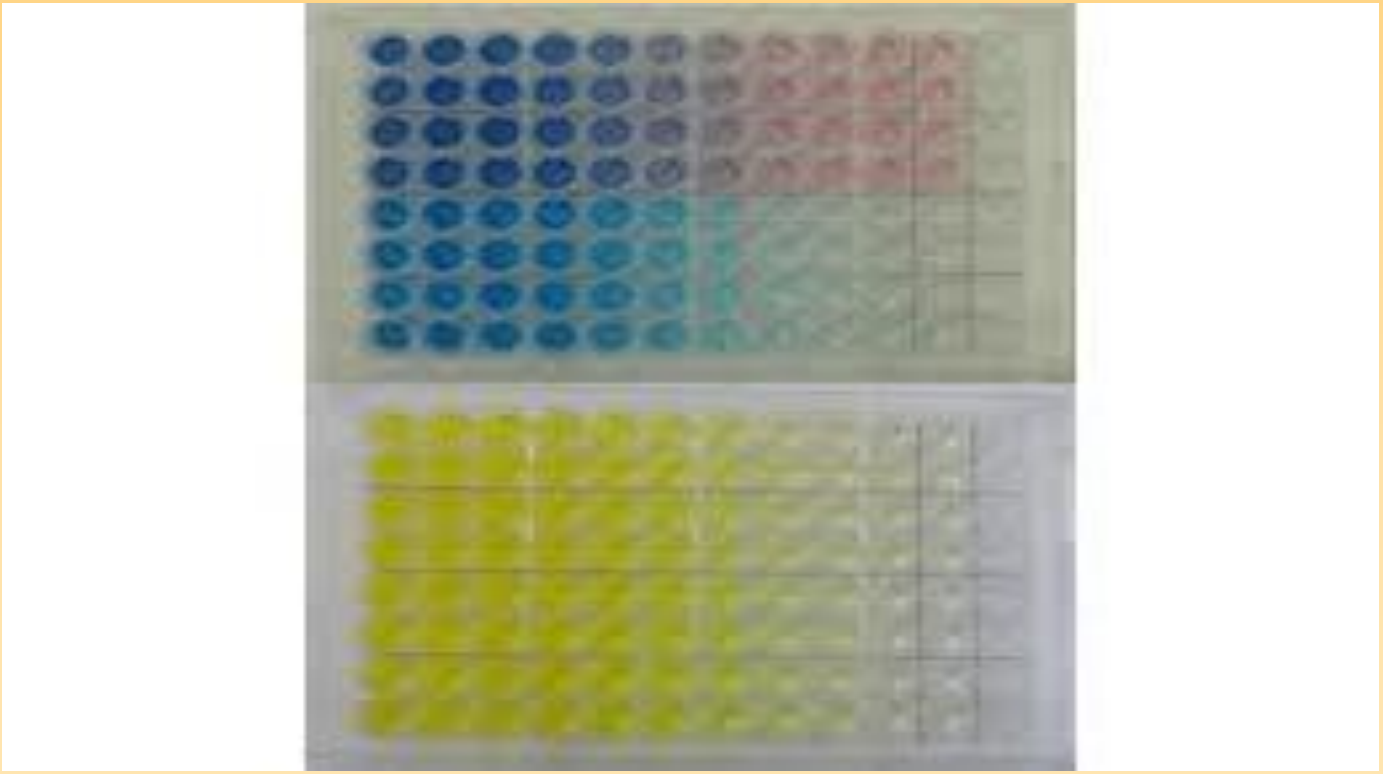
Immunization was performed with laminin-grown GBM stem enriched cells.

S to tehnologijo ugotovljeni proteomski biooznačevalci matičnih celic glioblastoma so še v postopku za objavo.

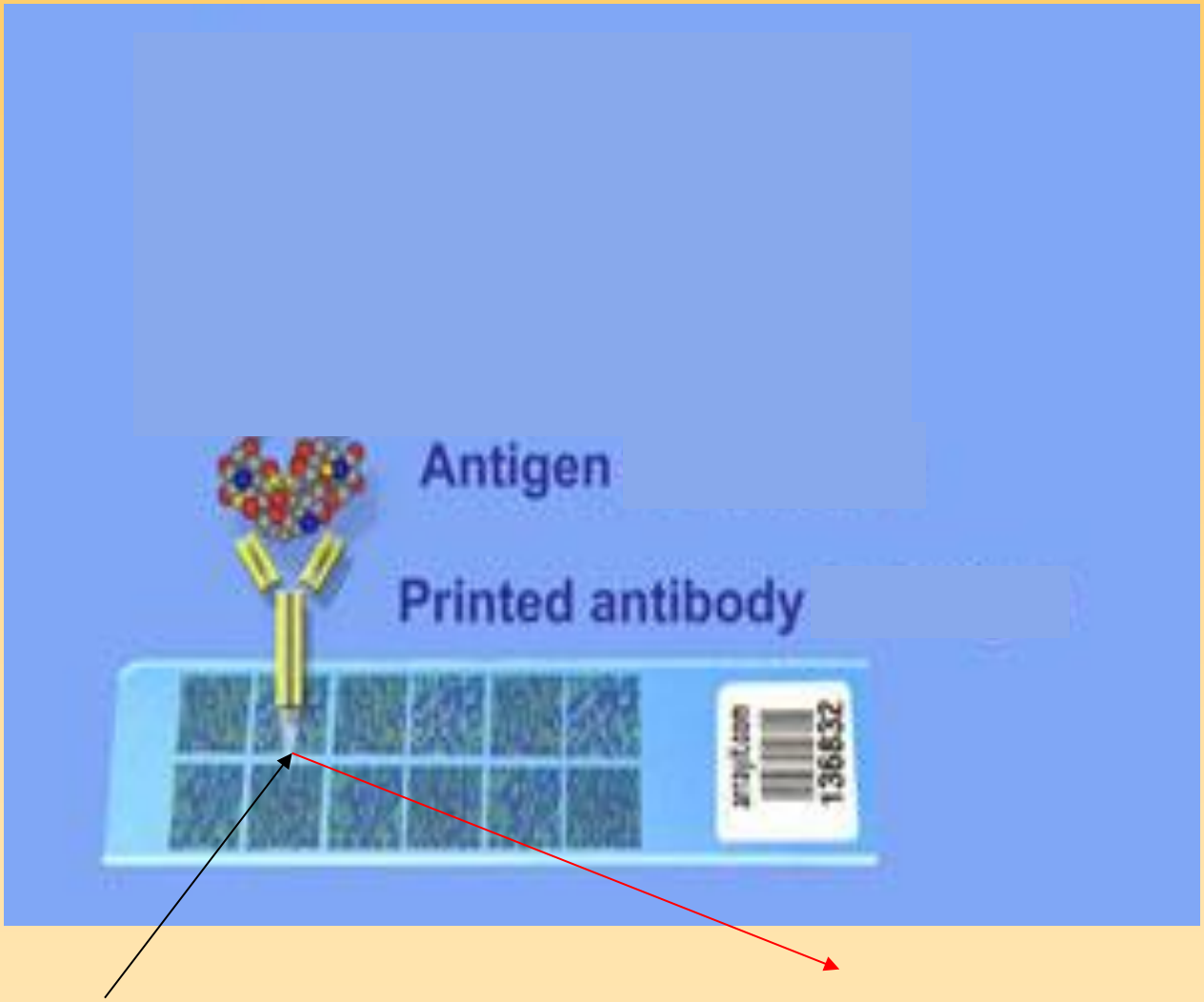


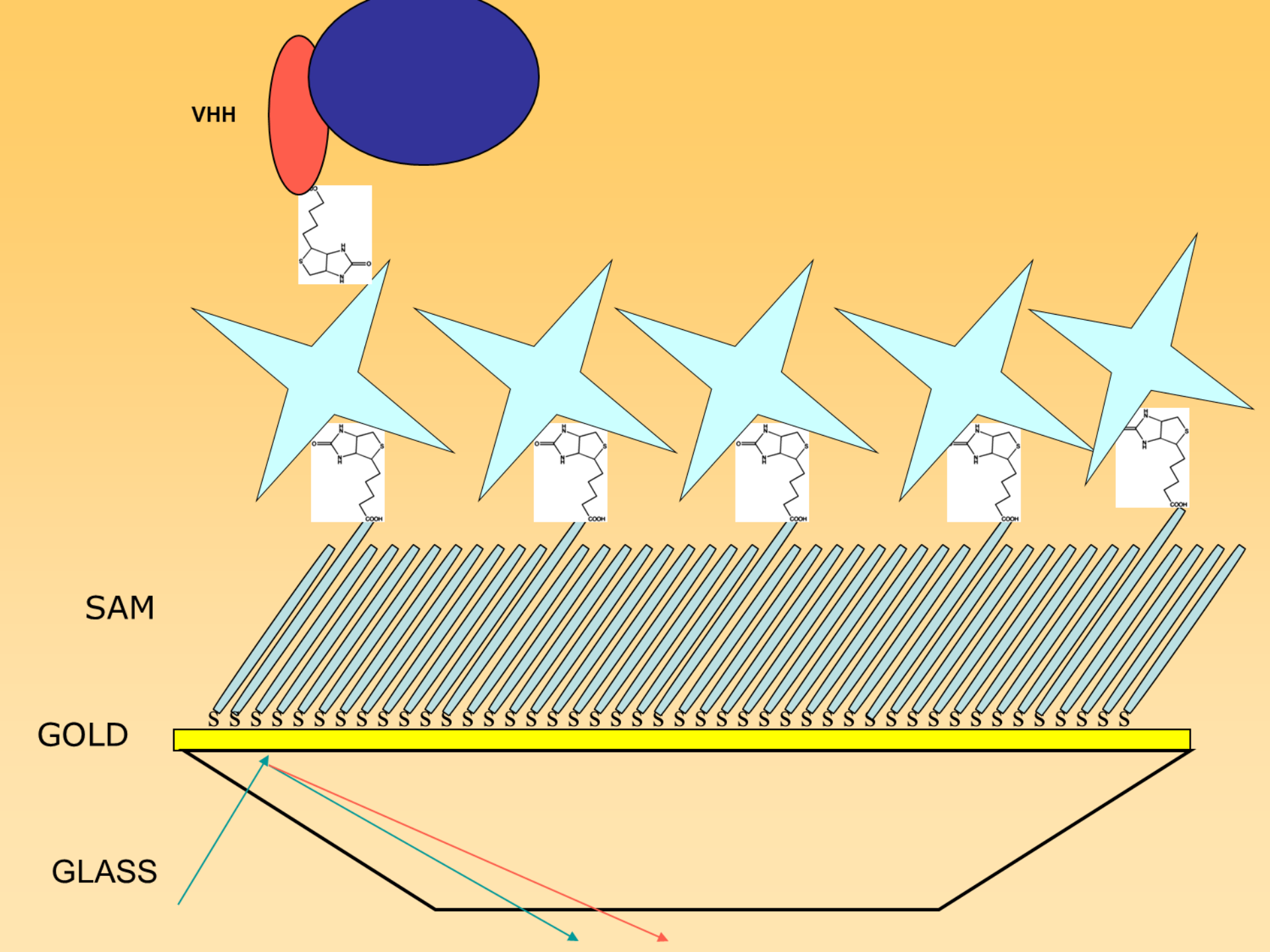
Razvoj multipleksnega testa ELISA z vezanimi ugotovljenimi specifičnimi nano-protitelesi: diferenčno barvanje proteinov iz rakastega in normalnega (referenčnega) tkiva.

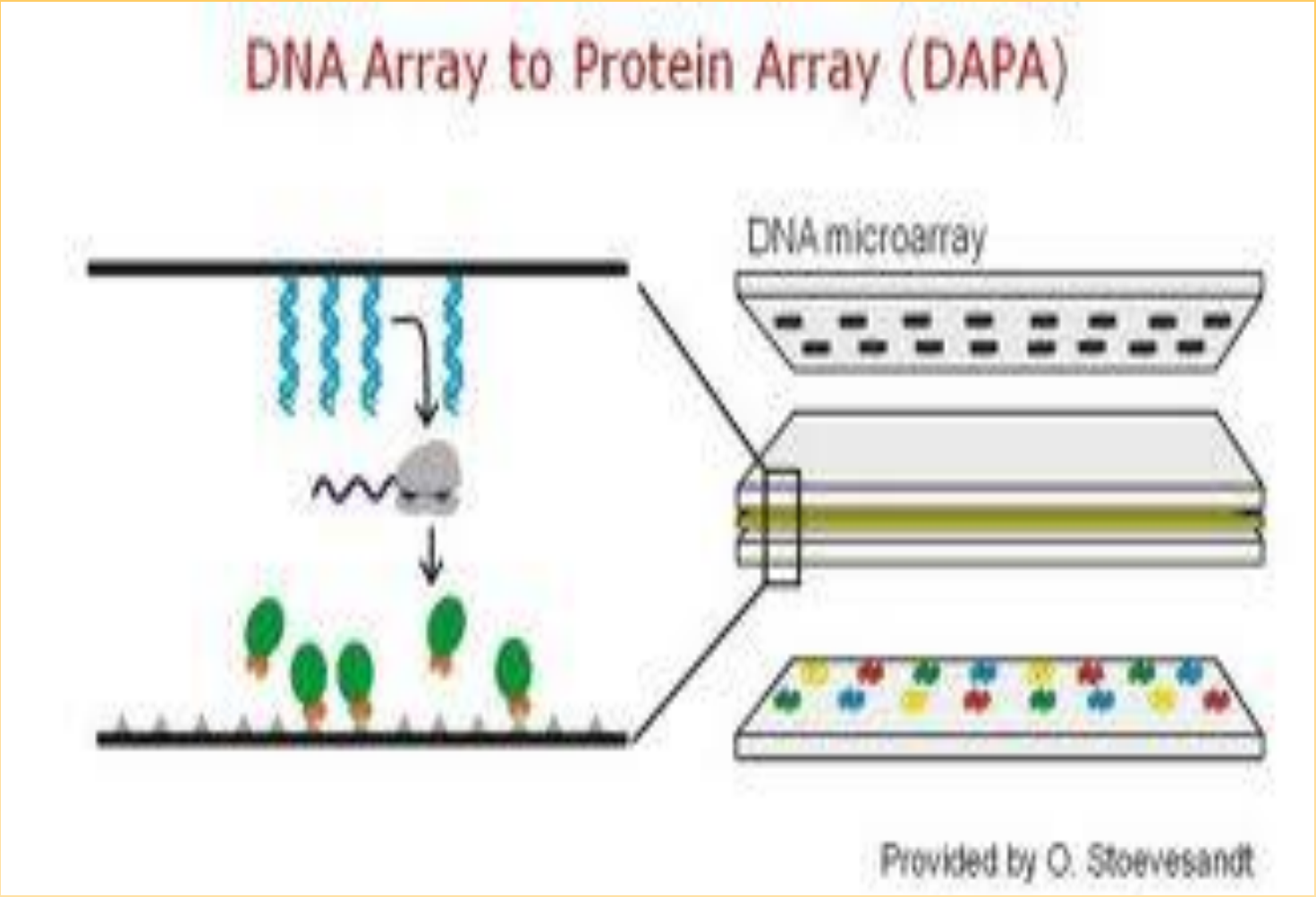
Za diagnostiko je zelo dobro, če ugotovljeni specifični biooznačevalec lahko najdemo tudi v telesnih tekočinah (kri, urin, cerebrospinalna tekočina).



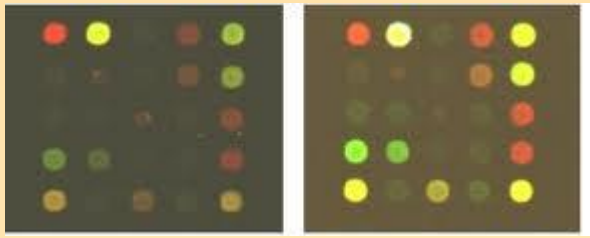
Razvoj diagnostičnega proteinskega bio-čipa z vezanimi nano-protitelesi.
Detekcija vezave: površinska plazmonska resonanca.




VHH
SAM
GOLD
GLASS



Razvoj diagnostičnega proteinskega biočipa z vezanimi nano-protitelesi na osnovi tehnologije DAPA.
Detekcija vezave: na osnovi diferenčnega barvanja proteinov.



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