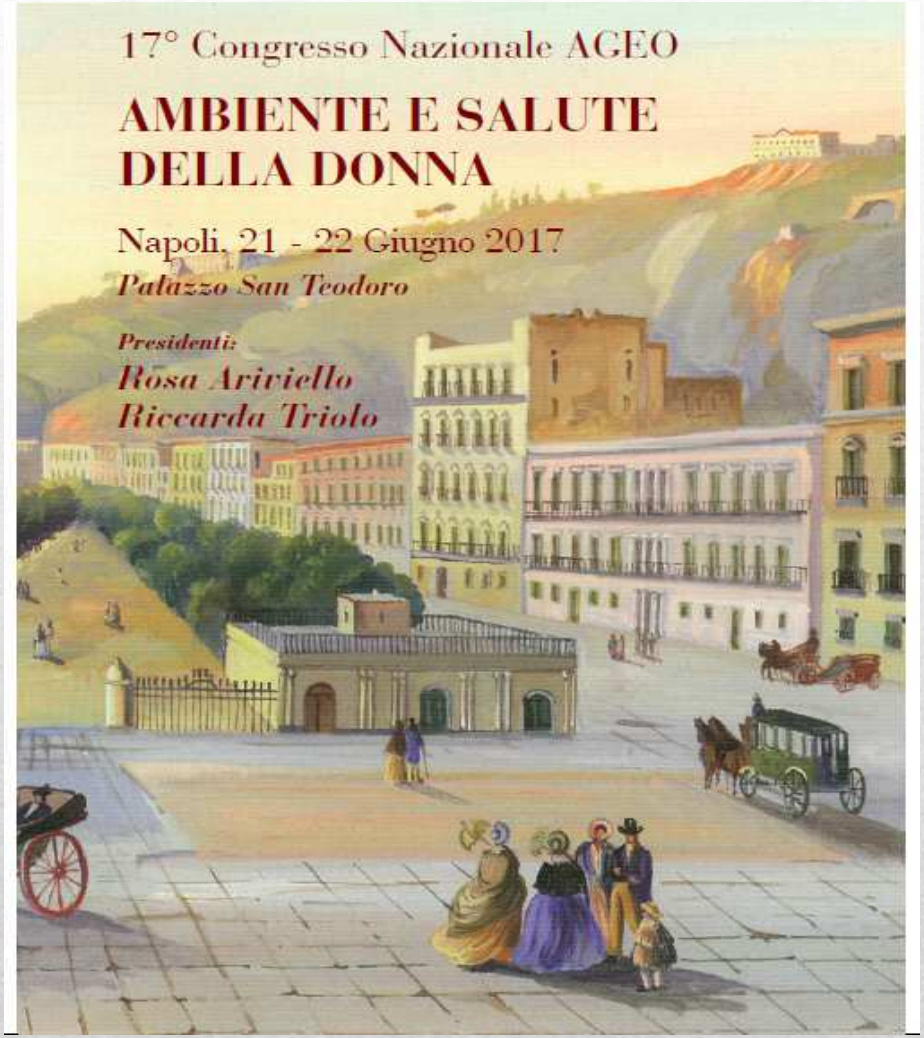
Genetica dei Tumori

Prof. Marina Di Domenico



Summary

Genome Landscapes and Tumorigenesis

- Clonal evolution and stochastic model
- Cancer stem cells model
- Cancer Risk Factors: genetic suscettibility
- Inflammation and cancer

Signalling Pathways in Human Cancer

- Oncogenic driver and tumour suppressor
- Oncogenes induces proliferation target proteins
- Hormone dependent pathways/refractory tumors

Genome editing and personalized medicine

- Baculoviruses as vectors for gene therapy against Human Cancer
- Cancer immunotherapy: Macrophages as cellular delivery
- Hormonal therapy, chemotherapy radiotherapy and surgery

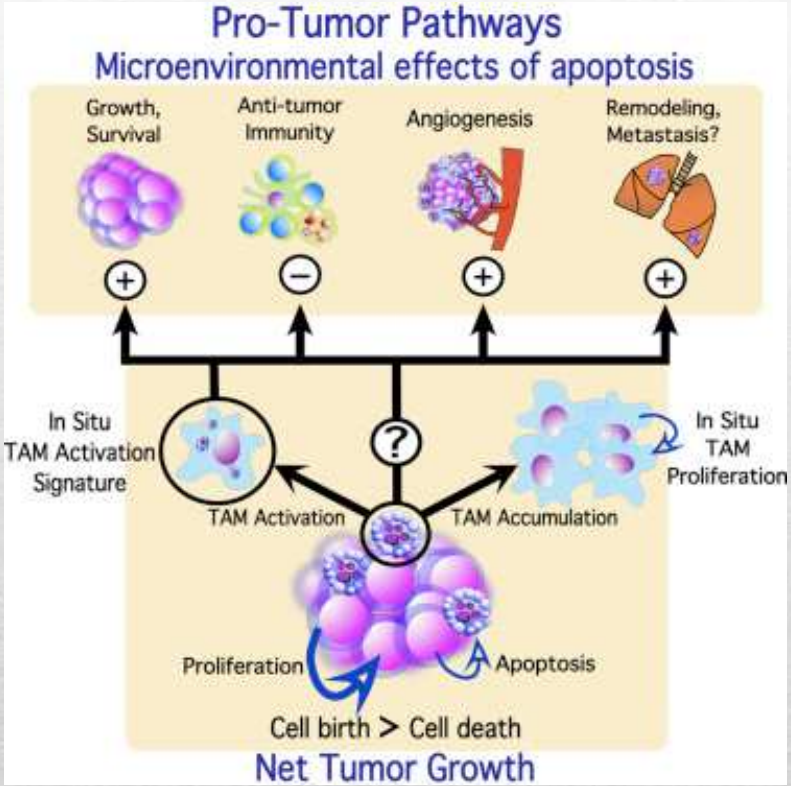
Study Methods: new approach

Future Perspectives and Conclusions

Genome Landscapes and Tumorigenesis

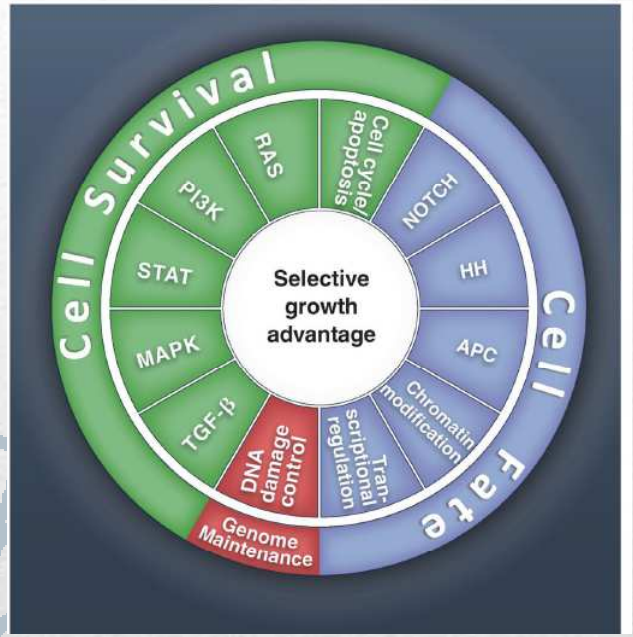
Most human cancers are caused by two to eight sequential alterations that develop over the course of 20 to 30 years.

Each of these alterations directly or indirectly increases the ratio of cell birth to cell death; that is, each alteration causes a selective growth advantage to the cell in which it resides.



Ford CA et al, Oncogenic Properties of Apoptotic Tumor Cells in Aggressive B Cell Lymphoma. Volume 25, Issue 5, p577–588, 2 March 2015
Vogelstein B et al, Cancer Genome Landscapes Science. 2013 March 29; 339(6127): 1546–1558.

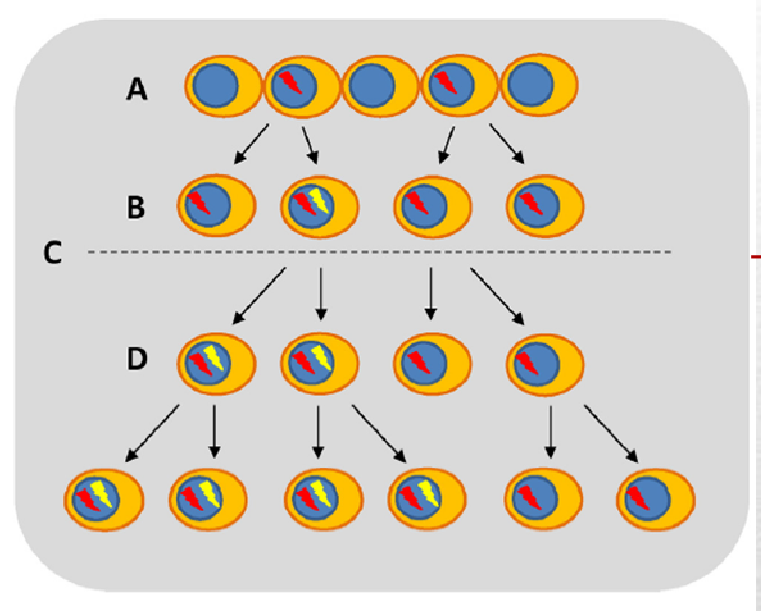
Genome Landscapes and Tumorigenesis



The evidence to date suggests that there are ~140 genes whose intragenic mutations contribute to cancer (so-called Mut-driver genes). There are probably other genes (Epi-driver genes) that are altered by epigenetic mechanisms and cause a selective growth advantage, but the definitive identification of these genes has been challenging.

Vogelstein B et al, Cancer Genome Landscapes Science. 2013 March 29; 339(6127): 1546–1558.

Clonal evolution and stochastic model



Micro-environmental change occurs, for example inflammatory stimuli or cancer treatment.

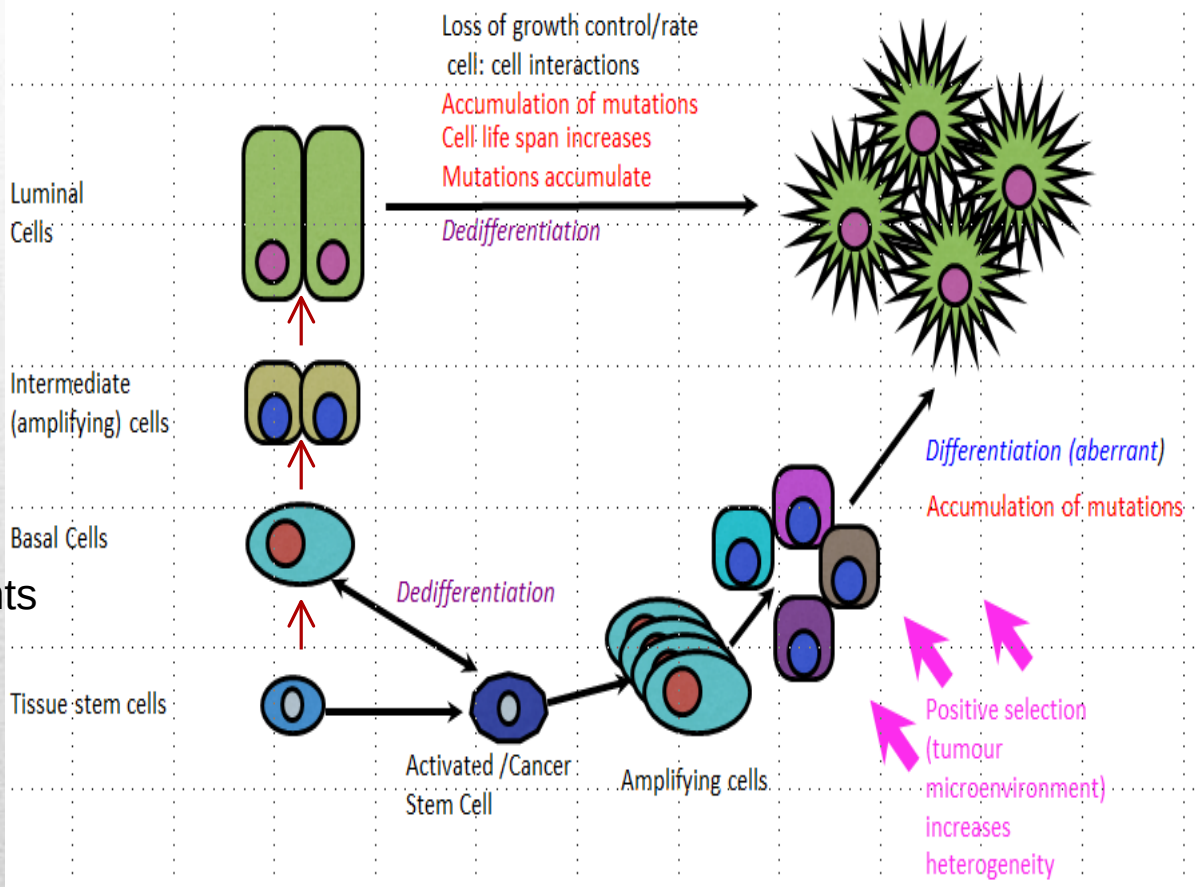
The known driver genes function through a dozen signaling pathways that regulate three core cellular processes: cell fate determination, cell survival, and genome maintenance.

Parker JR, et al. The molecular and cellular origin of human prostate cancer. Biochimica et Biophysica Acta 1863 (2016) 1238–1260

Cancer stem cells model

CSC hallmarks

- Can initiate new tumour growth
- Can differentiate from a primitive phenotype to produce the recognised phenotype of bulk tumour cells
- Responsible for tumour invasion and immunity escape
- Present in minimal residual disease after conventional treatments
- Responsible for tumour Recurrence after therapy
- Has a distinctive phenotype compared to the bulk of the tumour



Modified by Maitland and Collins, J Clin Oncol, 2008

Cancer isn't a single disease

The term cancer include more than 200 diseases all characterized by the uncontrolled proliferation of cells.

When the genetic material of a cell – the DNA – is damaged, mutations can arise, potentially disrupting normal growth and division.
An accumulation of mutations can turn normal cells into precancerous cells, which sometimes multiply and evolve into cancer cells. Cancer is a result of the accumulation of these cells.
Cancer is not an event, but a process that takes time, often years, to develop. The length of time varies widely and depends on the identity, order, and speed at which mutations accumulate.

American Association for Cancer Research website

Genetic Susceptibility

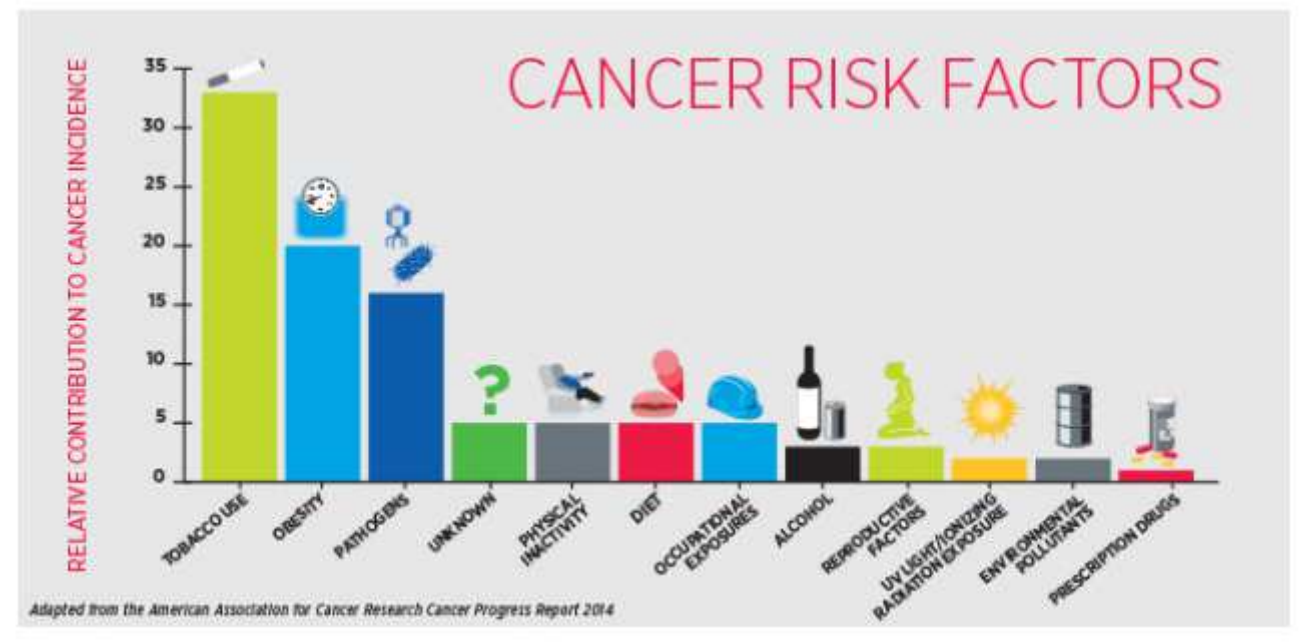
Genes contain the instructions necessary for a cell to work.
If some of these instructions are wrong, the **cell may not know what to do!**

DNA mutations may alter the genotype of an individual and lead to the cancer

Cancer Risk Factors

More than half of the 595,690 cancer deaths expected to occur in the United States in 2016 were related to preventable causes: including tobacco use, obesity, exposure to ultraviolet light, and vaccine-preventable infections with cancer-associated pathogens.

Adopting healthy lifestyles that eliminate or reduce the risk of recognized causes of cancer, could decrease the number of people diagnosed with many types of the disease.



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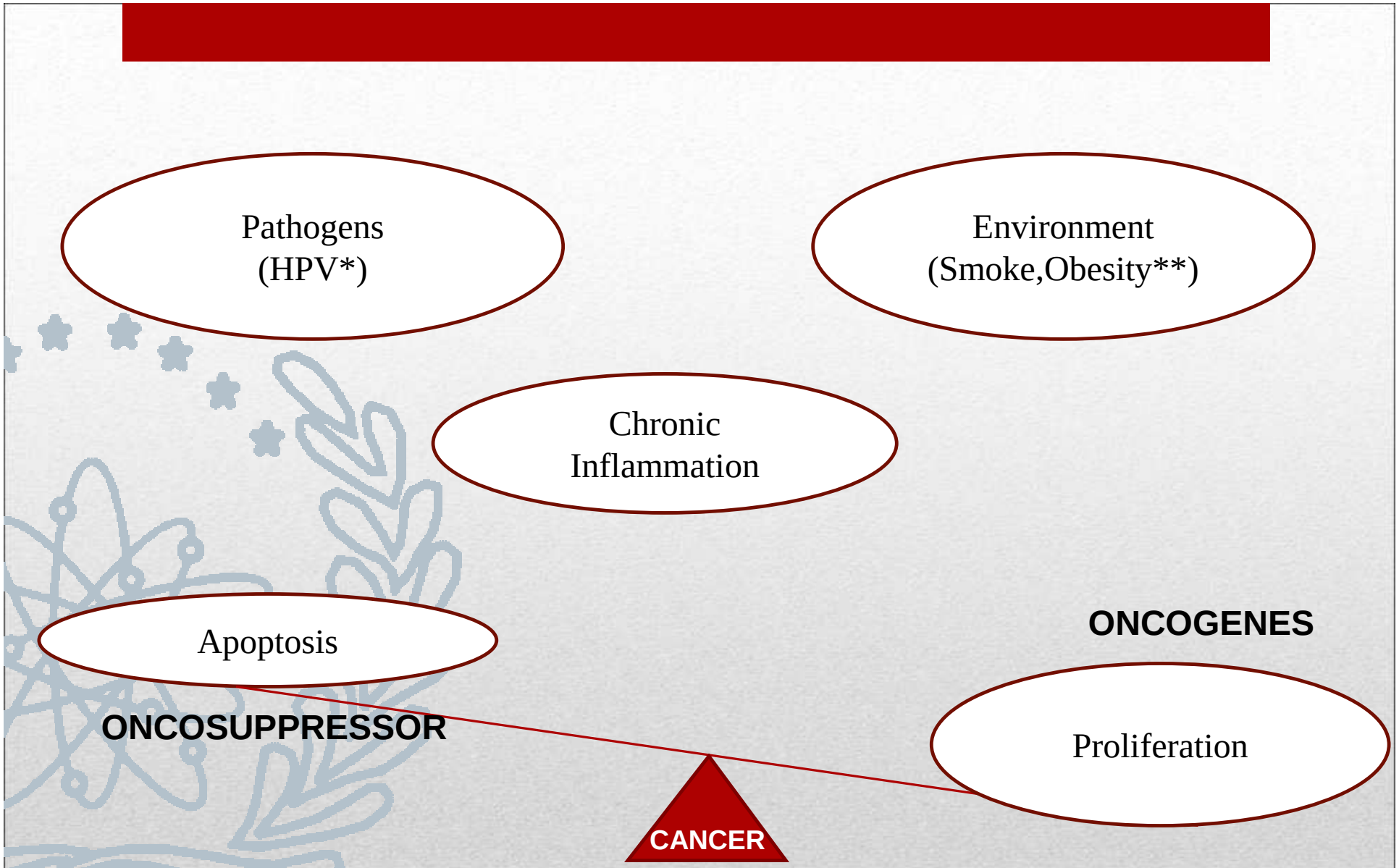
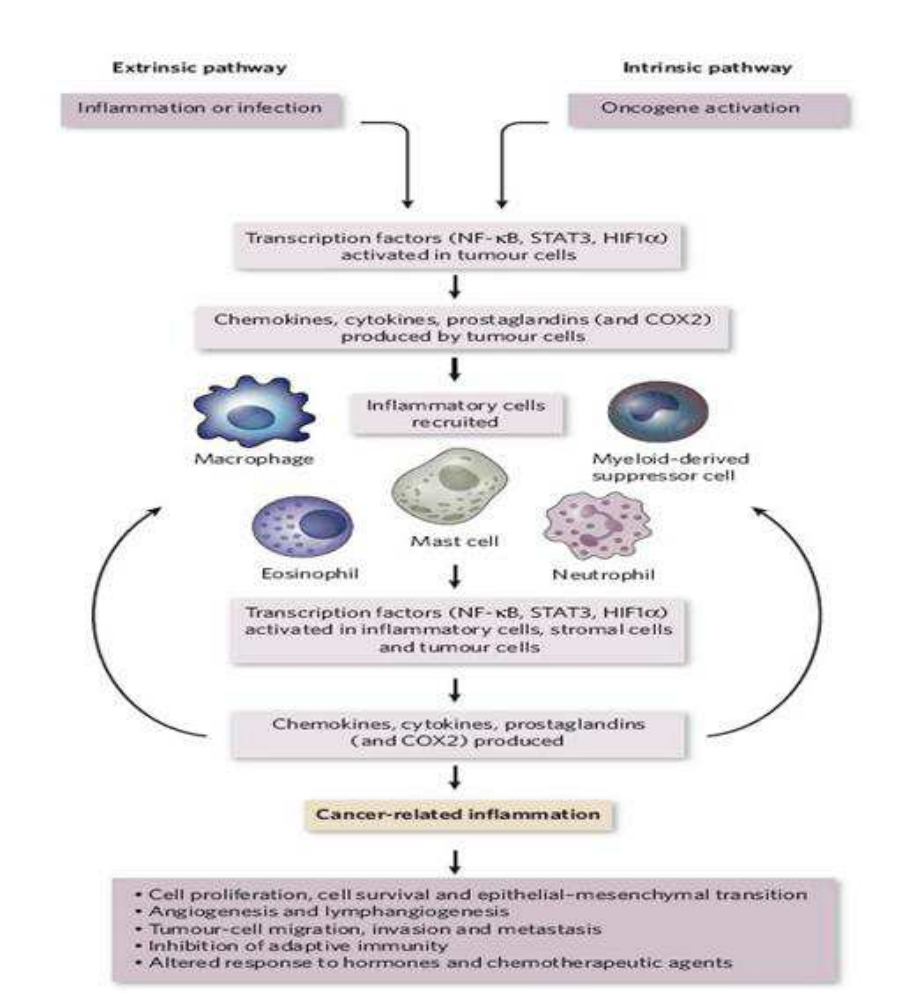


*HPV epigenetic mechanisms related to Oropharyngeal and Cervix cancers; Di Domenico et al; Cancer Biol Ther. 2017 Mar 31:0.
**Multifaceted Breast Cancer: The Molecular Connection With Obesity.; Di Domenico et al; Cell Physiol. 2017 Jan;232(1):69-77.

Inflammation and Cancer



*Alberto Mantovani, et al. Nature **454**, 436-444 2008; Cancer-related inflammation*

Inflammation and Cancer

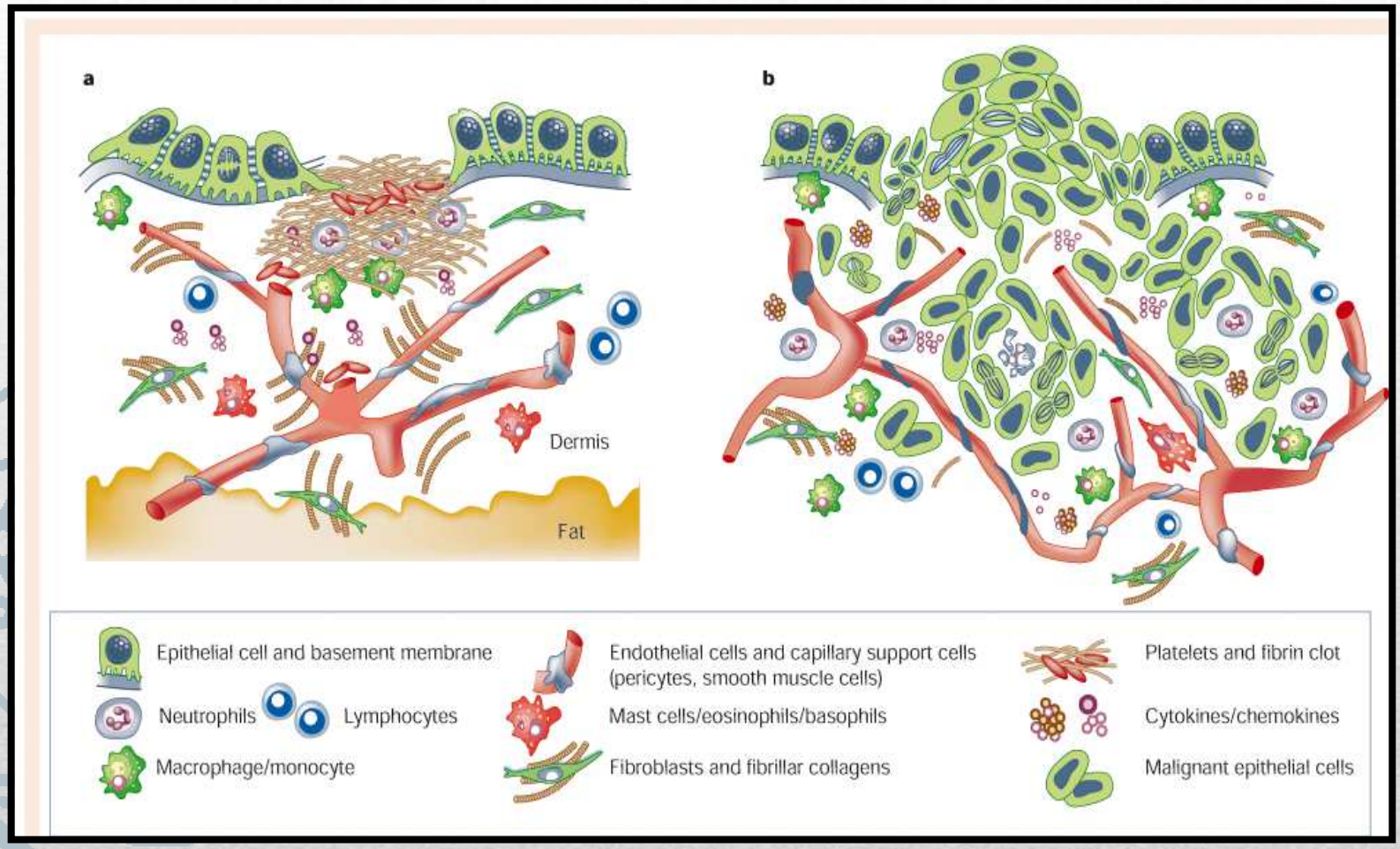
Role of Inflammation in Cancer Growth and Development

What is inflammation?

- Inflammation is considered a protective response intended to eliminate the initial cause of cell injury
- Inflammation is a crucial function of innate immune system that protects against pathogens

Inflammation and Cancer

Inflammation Neoplasia



Coussens L.M.,Werb Z. Nature 2002

Inflammation and Cancer

Cytokine and chemokine balances regulate neoplastic outcome

Altered balance of pro or anti inflammatory cytokines is correlated with:

- High levels of monocytes
- Neutrophil infiltration

…leucocytes inflitration is associated with

1. Angiostasis
2. Presence of TAM (tumor associated macrophages)

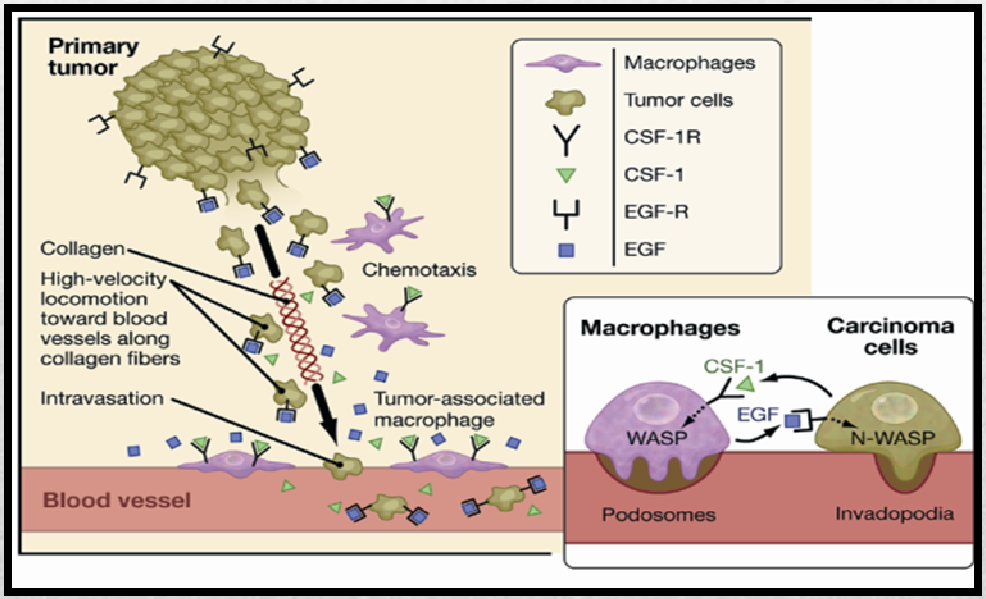
Coussens L.M.,Werb Z. Nature 2002

Inflammation and Cancer

Why are macrophages a key element in inflammation and neoplastic progression ?

Because they respond to microenvironment signals with polarized **genetic** and functional programs

- They capture antigens and after maturation migrate to lymphonodes to stimulate T-lymphocites activation
- Soluble factors such as IL-6 e CSF-1 derived from neoplastic cells



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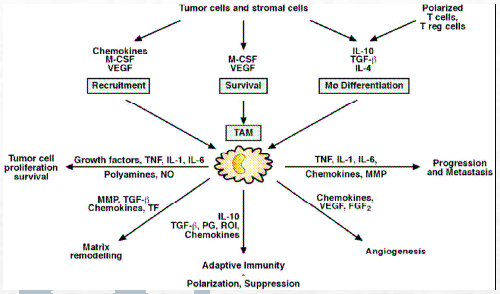
Inflammation and Cancer



Pro – tumorigenic functions of TAM

- TAMs migrate to hypoxic tumor areas where stimulate angiogenesis by expressing factors such as VEGF and ANG1
- TAMs recruit other haematopoietic cells
- TAMs promote tumor invasion by producing proteases (MMP9) and cathepsins that break-down the basement membrane and remodel the stromal matrix
- TAMs produce MMP9 that contributes to angiogenesis
- EGF, TGFbeta, IL-8 and TNFalpha contribute to migration of tumor cells towards vessels and provide proliferative and anti-apoptic signals to these cells

Jeffrey W. Pollard Nature review 2004

Inflammation and Cancer

- The functional relationship between inflammation and cancer is not new
- In 1863 Virchow hypothesized the origin of cancer was at sites of chronic inflammation based on this hypothesis: same classes of irritants and the tissue injury enhance cell proliferation.

L.M. Coussens and Z.Werb nature 2002

Table 2. Inflammatory conditions that predispose to cancer

Malignancy	Inflammatory stimulus
Bladder cancer	Schistosomiasis
Gastric cancer	*H. pylori*-induced gastritis
MALT lymphoma	*H. pylori*
Hepatocellular carcinoma	Hepatitis virus (B and C)
Kaposi's sarcoma	HHV8
Bronchial carcinoma	Silica
Mesothelioma	Asbestos
Bronchial carcinoma	Asbestos
Ovarian cancer	Salpingitis/talc/ovulation/endometriosis
Colorectal cancer	Inflammatory bowel disease
Oesophageal cancer	Barrett's metaplasia
Papillary thyroid carcinoma	Thyroiditis
Prostate cancer	Prostatitis

Smoldering and polarized inflammation in the initiation and promotion of malignant disease
Frances Balkwill, Kellie A. Charles, and Alberto Mantovani. Cancer cell: March 2005

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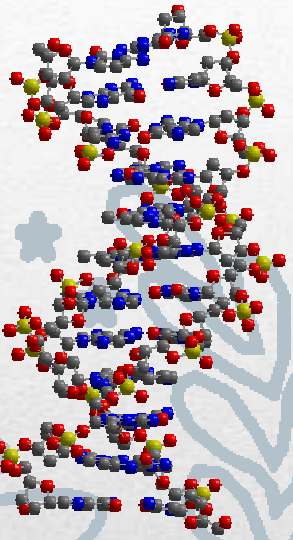
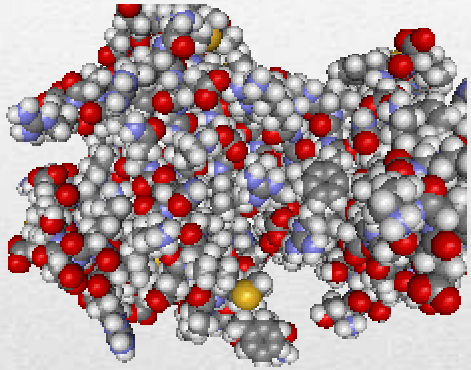
Oncogenic induces proliferation target proteins





DNA → mRNA → Protein

transcription
Oncogenes and oncosuppressor

translation
Proliferation target proteins

Oncogenic drivers and tumour suppressor

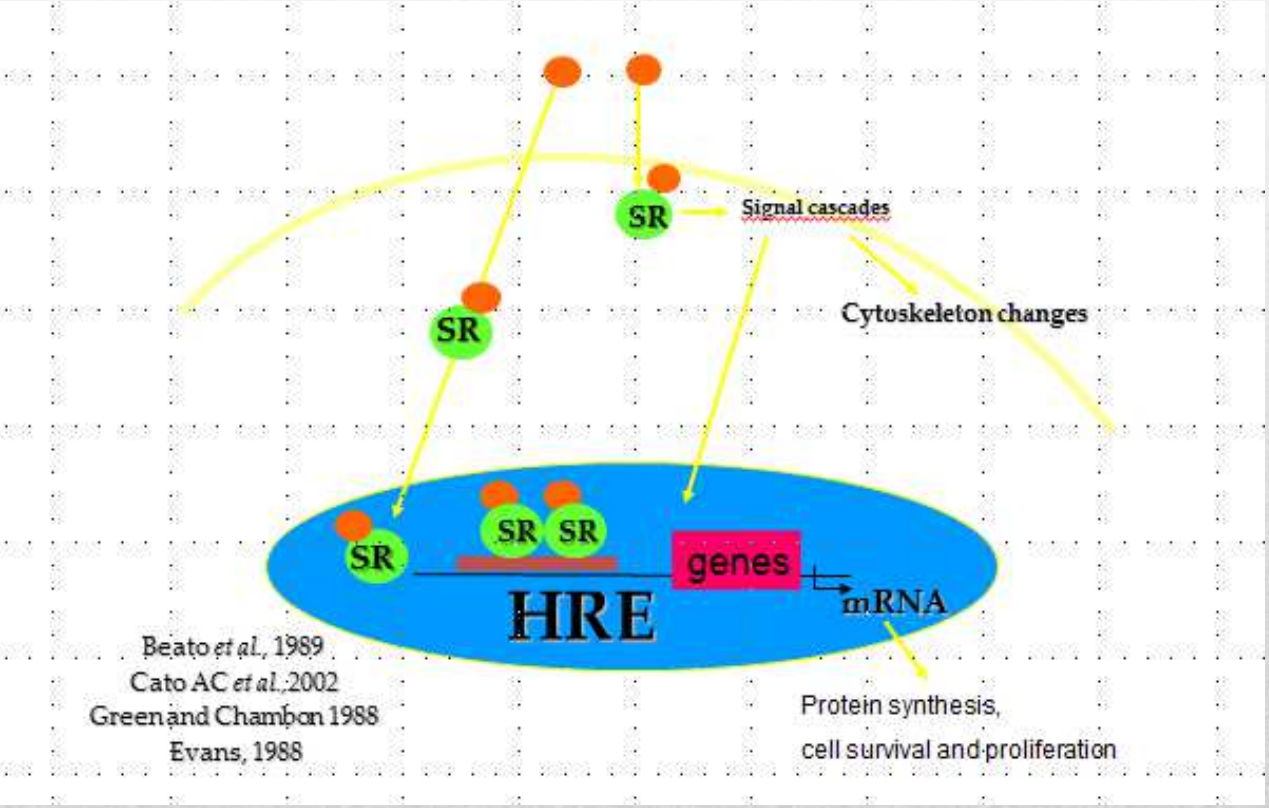
Molecular defects which can drive primary prostate cancer.

Primary prostate cancer molecular defects	
Oncogenic drivers	**Tumour suppressors**
TMPRSS2-ETS fusions A translocation or interstitial deletion event places an ETS factor, most commonly ERG but also ETV1, ETV4 and ETV5, under the control of the TMPRSS2 promoter. The ETS proteins are developmental transcription factors that affect proliferation, migration and transformation of cells. These gene fusions are seen in over half of all prostate cancers.	PTEN deletion Heterozygous deletion of the PTEN gene is observed in ~40% of primary tumours. This causes haploinsufficiency of the gene product; PTEN is the reciprocal phosphatase of PI3K. Reduced removal of PI3K phosphorylations causes unchecked AKT activation that allows for increased cell survival and proliferation. Mutation of the gene is also observed.
MYC overexpression MYC is seen to be upregulated in early prostate tumours and its locus is frequently amplified in advanced cancers. The gene encodes a transcription factor that has well characterised transformative properties due to its role in cell cycle progression.	CDKN1B deletion CDKN1B is deleted in ~20% of primary tumours. The protein acts is a CDK inhibitor that controls the G_1 cell cycle checkpoint, loss of the protein allows for easier commitment to the cell cycle and thus promotes increased proliferation. Mutation of the gene is also observed.
Telomerase activation The protection of chromosome ends by inappropriate activation of telomerase prevents replicative senescence in highly proliferative cancer cells. Telomerase expression is switched occurs during hgPIN and early prostate cancer.	NKX3.1 deletion NKX3.1 is heterozygously deleted in up to 85% of prostate cancers and is downregulated in PIN. The protein is a homeobox transcription factor that regulates prostate epithelial development.
IL-6 addiction Inflammation causes upregulation of both IL-6R and oncostatin M, an IL-6 signal transducer, in the prostate stem cell population. An increase of systemic IL-6 in prostate cancer patients creates a positive feedback loop favourable towards transformation through enhanced STAT3 activation and associated downstream gene expression.	SPOP mutation SPOP is mutated in 6–15% of primary cancers. This is a molecular event that is mutually exclusive of TMPRSS2-ERG fusions. SPOP is an adaptor protein for the Cullin3 E3 Ubiquitin Ligase and directs the proteasomal degradation of oncogenic proteins such as AR, DEK and ERG, and is also involved in the DNA damage response and cellular senescence. Mutations cluster in the substrate binding domain of the protein and abrogate SPOP effectiveness.
	CHD1 deletion CHD1 is commonly deleted in primary tumours, often coincides with SPOP mutation, and is mutually exclusive from the TMPRSS2-ERG fusion. The protein is involved in chromatin remodelling.

Parker JR, et al. The molecular and cellular origin of human prostate cancer. Biochimica et Biophysica Acta 1863 (2016) 1238–1260

Oncogenes induces proliferation target proteins

During tumorigenesis, in which pathways are involved the proteins encoded by Oncogenes?



Steroid hormones are involved in different processes

The classic model of steroid transcriptional action is not enough exhaustive to explain steroid action on cell proliferation. Therefore, we needed a new model on which basing the future analysis of the proliferative action of steroids (Gorski,1997)

Different hypotheses have been proposed to explain cell proliferation:
-Steroids might act through interaction of their receptors with specific DNA sequences regulating the expression of genes required for cell multiplication (Weisz and Bresciani 1993; Loose-Mitchell 1988)

-Production of polypeptide growth factors by the target cells (Dickson and Lippman,1987) or other mechanism

Steroid hormones are involved in different processes

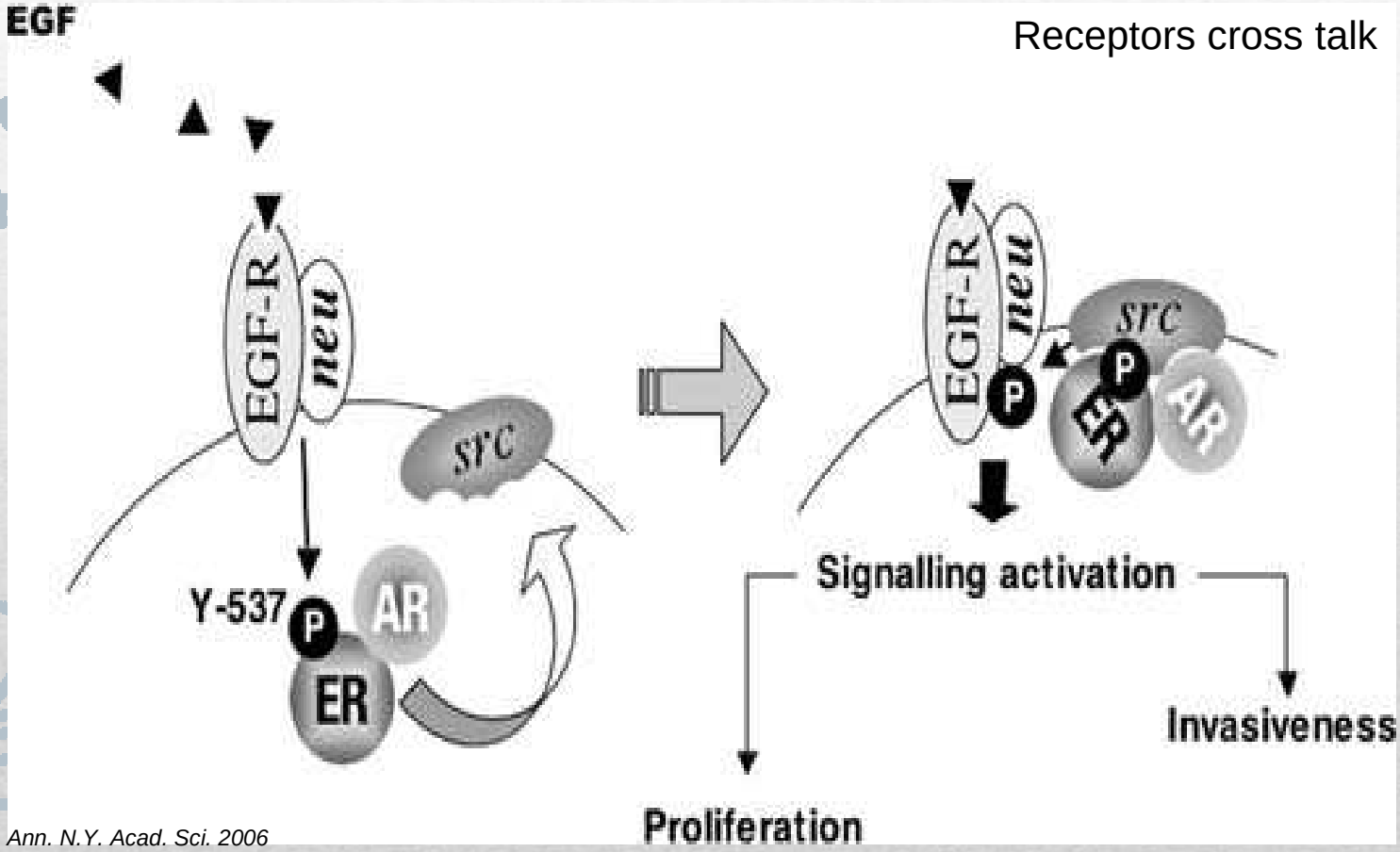
- Cell proliferation
- Tissue differentiation
- Morphogenesis

- Programmed cell death
- Neoplastic transformation and progression
- Homeostasis

Proliferative role of signalling pathway activation by steroids

Fantastic history of steroid receptors ….

A new link between epidermal growth factor (EGF) signaling and extranuclear steroid receptors in LnCap



Migliaccio A. et al. Ann. N.Y. Acad. Sci. 2006

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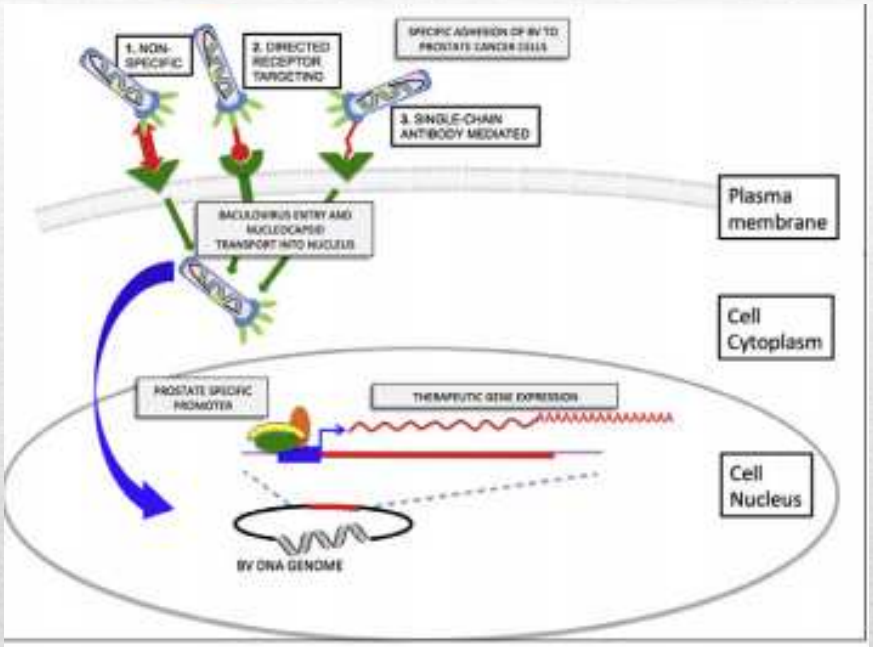
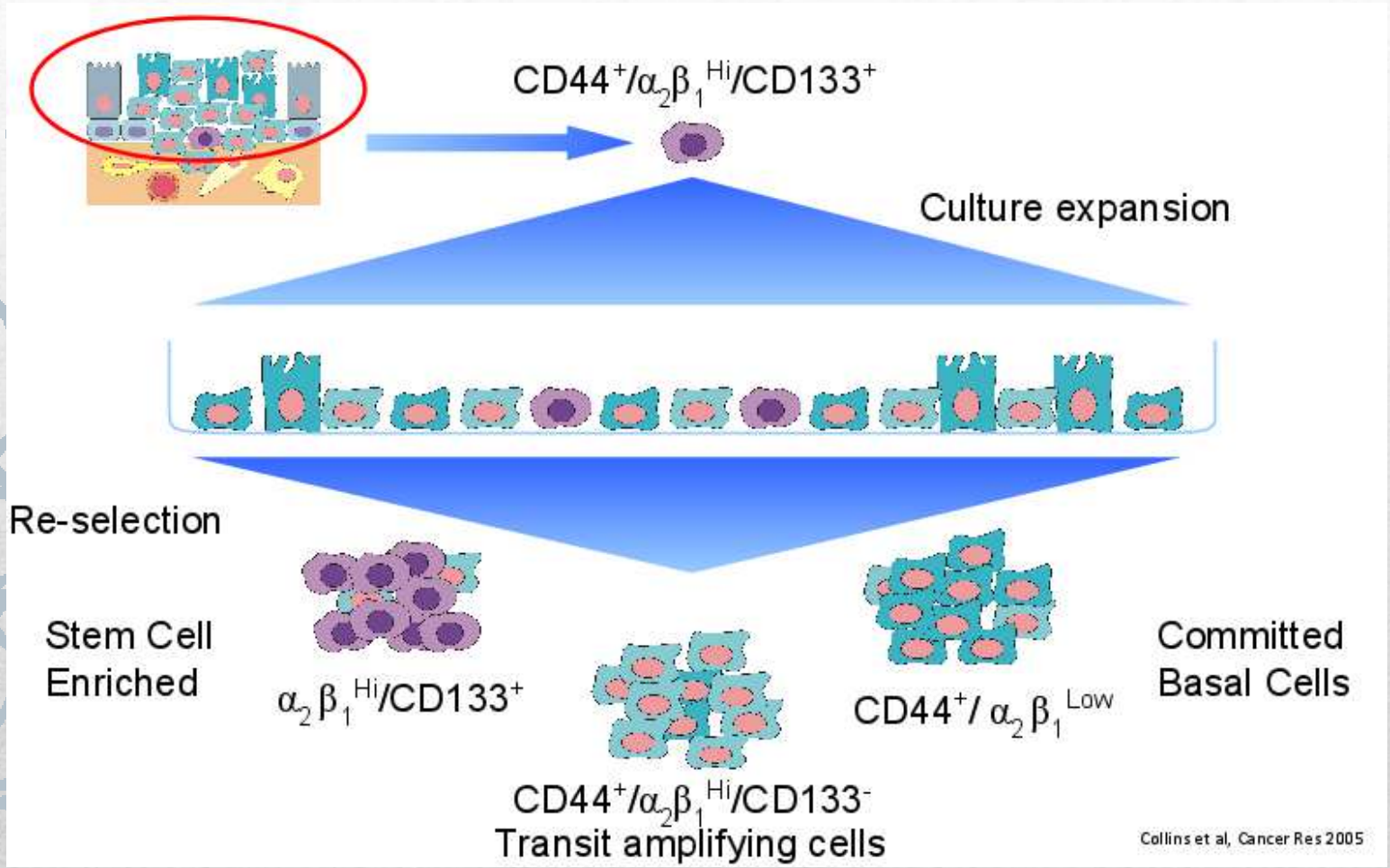
Baculoviruses as Vectors for Gene Therapy against Human Cancer

The natural ability of Bv to preferentially infect cancer cells can be used on its own (1. nonspecific entry), which can be enhanced
(2) by the use of fusion proteins or peptides (directed receptor targeting) and
(3) single chain antibody targeting cell surface proteins.

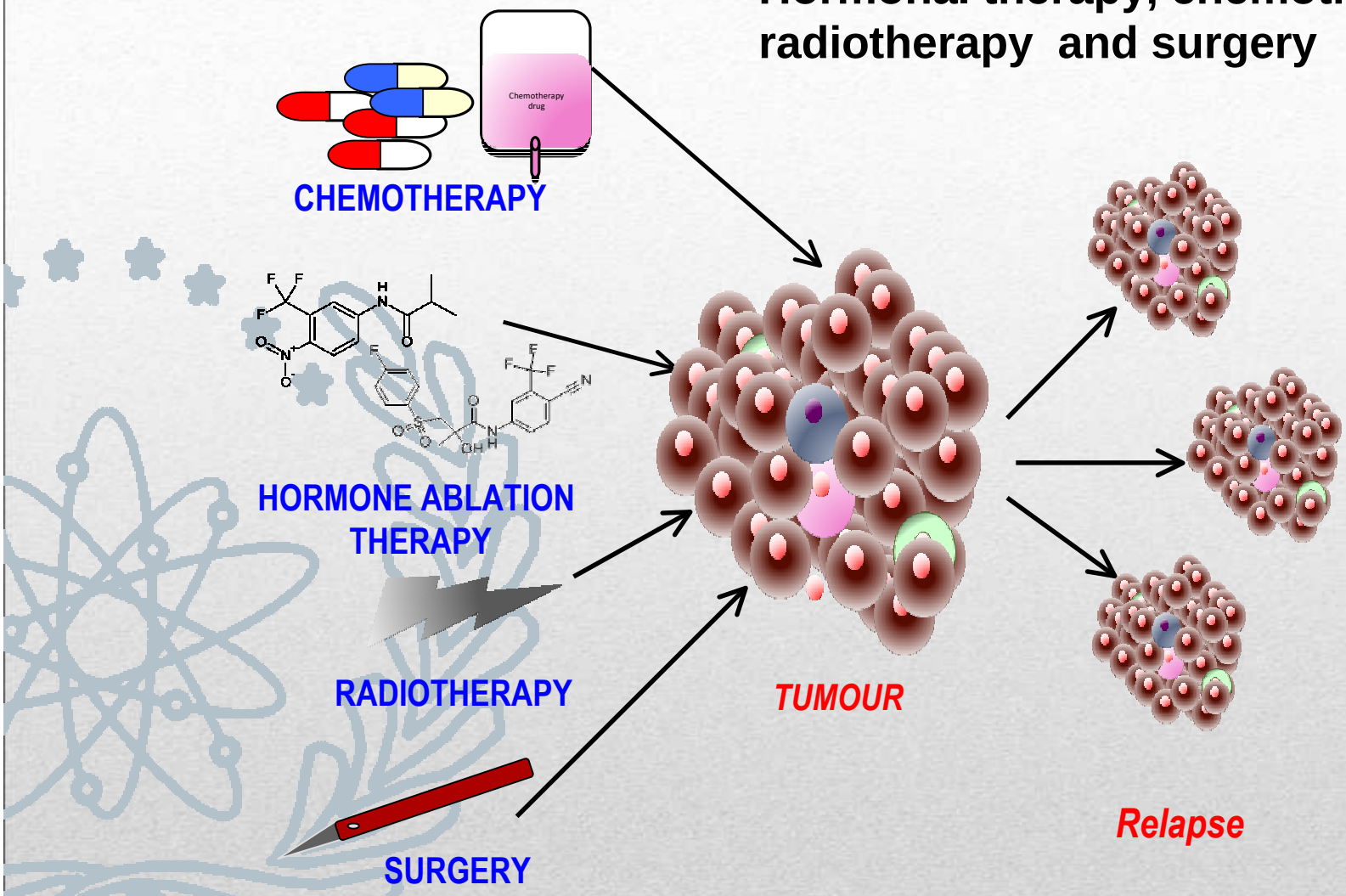


Lindsay J. Stanbridge et al. Baculoviruses as Vectors for GeneTherapy against Human Prostate Cancer Journal of Biomedicine and Biotechnology • 2003:2 (2003) 79–91

Cancer immunotherapy : strategy to identify useful immune cell target agents



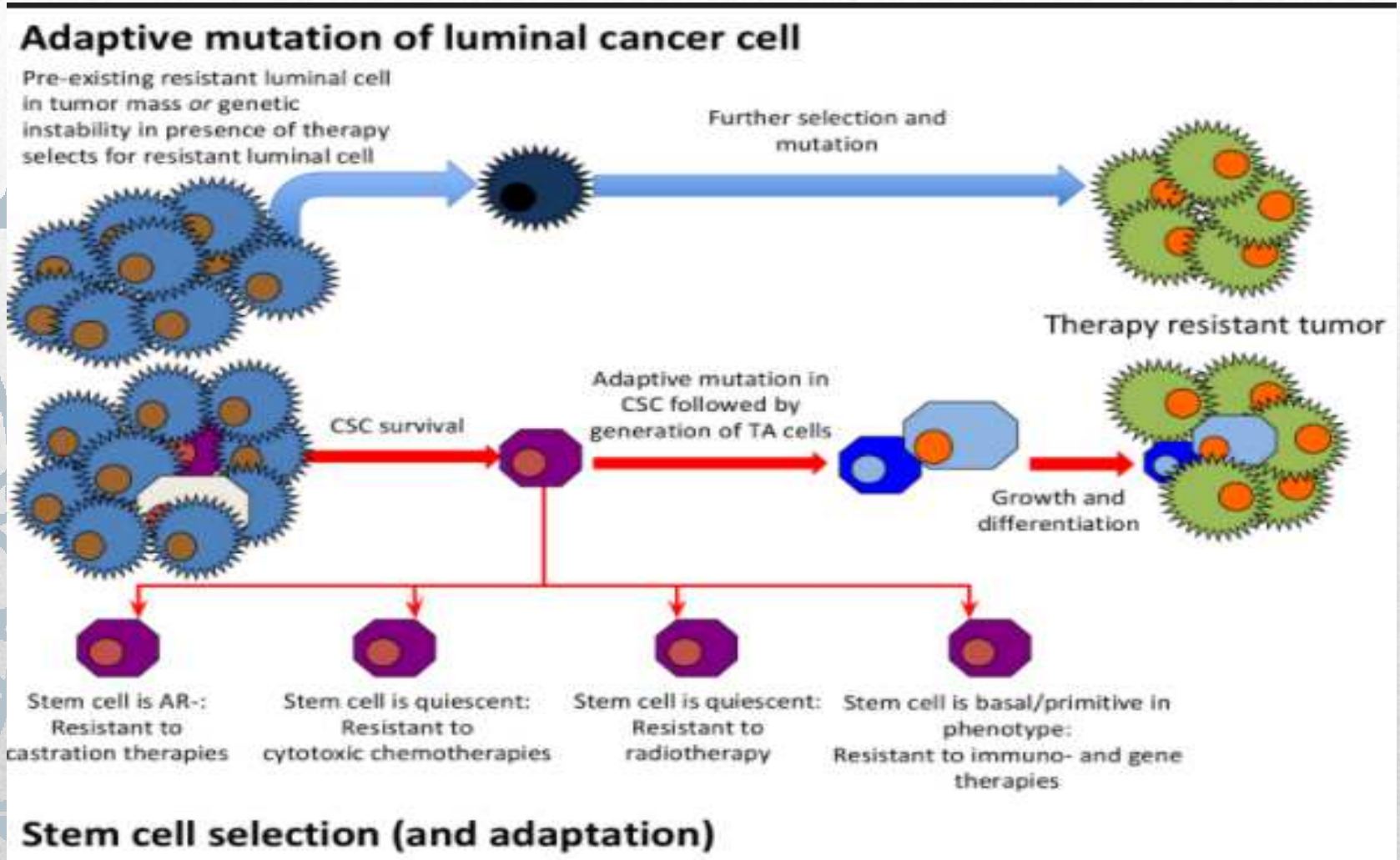
Hormonal therapy, chemotherapy, radiotherapy and surgery



Modified by Maitland and Collins, J Clin Oncol, 2008

Which are the consequences of these therapies in a heterogeneous neoplasia?

Hormone Refractory Tumors and Resistant



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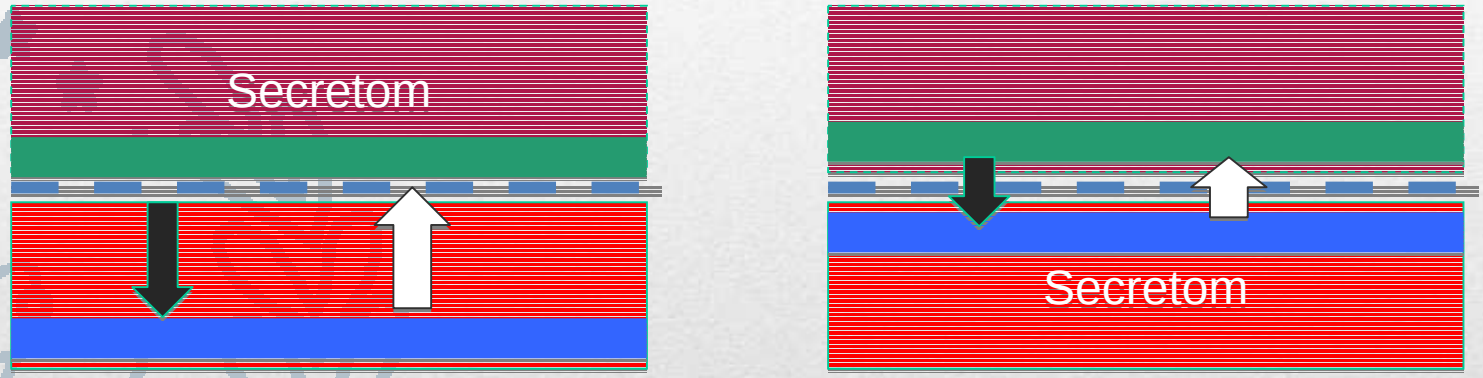
Co-Cultures: Cell-Cell Interaction (paracrine regulation)

Cultivation of 2 different cell types which are mechanically separated by a porous membrane (0.4 μm)



Soluble molecules secreted out from each cell (up to 30% of all proteins) can be analysed conferring to their influence on the neighboured cell type.
Functional/biochemical analysis possible but requires experience!

But:

Standard MTP-Tests (WST, BrdU, LDH) can be adapted!
Identification of secreted molecules is restricted to MOI

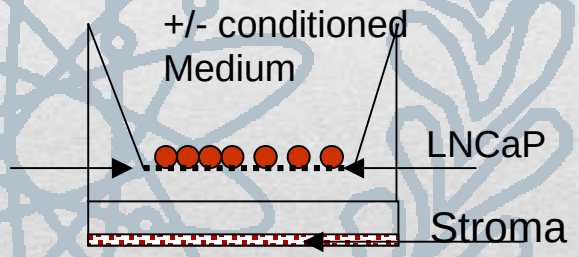
Modified by Feola A et al, J Cell Biochem. 2013 Sep;114(9):2114-9. doi: 10.1002/jcb.24558.

Verification of CAF-mediated functions

Co-culture as one tool to proof CAF-TC crosstalk

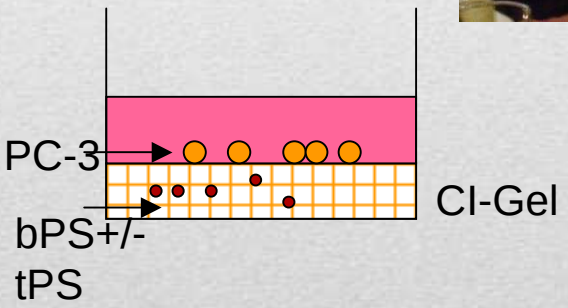
*Norman Maitland attending our annual AuF Meeting - Berlin 2012

Lang&Maitland



Stroma enhanced proliferation of Tumor cells (XTT)

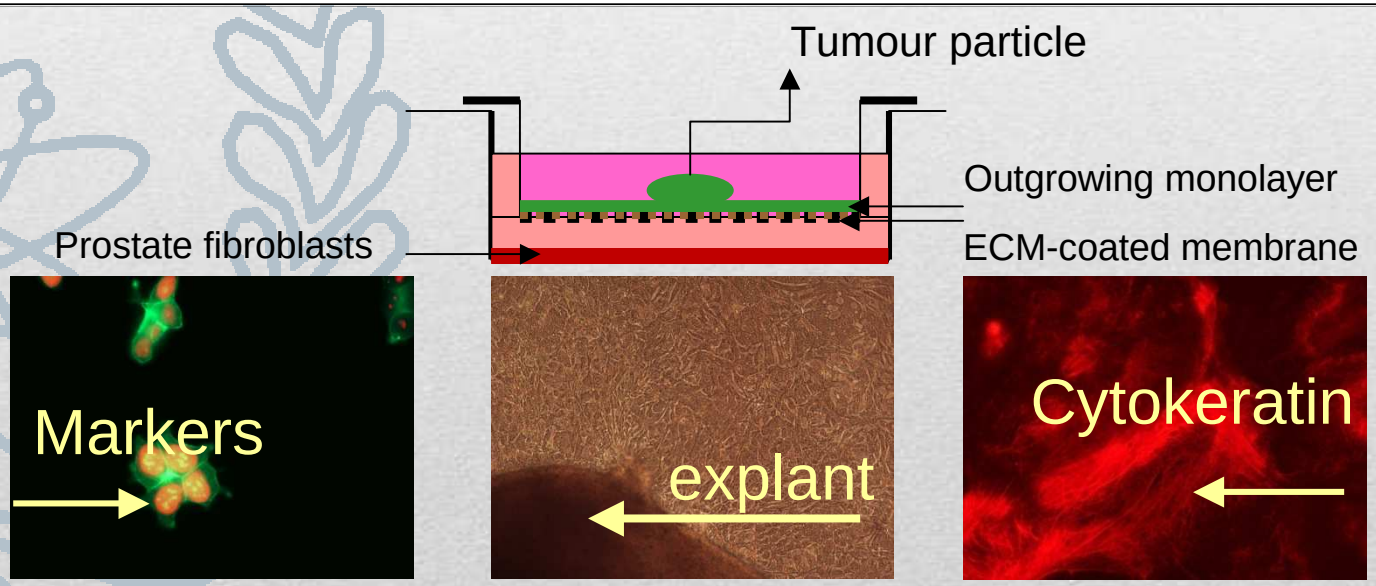
Lang



Lang SH, et al. Cell Growth Differ. 2001 Dec;12(12):631-40.

Co-culture with tumor derived fibroblasts as a promising approach

- Transfer the organ immediately after surgery to the pathology
- Remove specimens as donor matched pairs with the aid of an expert pathologist
- Transfer specimens (sterile) immediately to the cell culture lab (SOP)
- Use a coculture system with prostate fibroblasts in the companion plate
- Place tumour(normal) specimen onto ECM-coated membranes with 0.4 µm porous
- Combine both systems after 24 hrs
- Use PrEBM medium in the upper part (insert) and DMEM in the companion plate



Modified by Saar A. Urologe A. 2011 Aug;50(8):961-7. doi: 10.1007/s00120-011-2630-7. German.

What is your interest? Research? – Screening?

Strategies to overcome the drawbacks of flat cell biology

(Two) different approaches in 3-D cell biology

Screening

Permanent cell lines
Cells not representative

- Formation of spheroids
- Mimicking of drug delivery
- Simulating cell-cell interactions
- Combination with ECM
- Screening systems available
- highly reproducible
- not limitations with respect to material
- partial improvement
- genetic engineering (e.g. GFP)

Research

Patients material
Contains all cells of interest

- Limited material
- Highly heterogeneous
- limited lifespan
- Selection during cultivation
- Fast shift in gene expression
- Reduced tumorigeneity in mice
- Microenvironment artificial
- No HTS possible
- Characterization difficult

Marked Differences between these experimental approaches:!

Modified by Saar A. Urologe A. 2011 Aug;50(8):961-7. doi: 10.1007/s00120-011-2630-7. German.

3D-tissue culture models in (prostate) cancer biology

Systems in use
(CL = cell lines; PC= Primary cultures)

- Co-cultivation CL-PC
- Spontaneous aggregation CL-PC
- Liquid overlay CL
- Scaffold based cultures CL
- Spinner flasks-Shaker-Roller CL
- Microcarrier beads CL
- Pre-engineered composite scaffolds CL
- Xenograft transplantation CL-PC
- Matrigel/Collagen CL-PC
- Synthetic matrix (PLA) CL-PC
- Soft agar CL
- Bioreactors CL
- 3D-assymetric collagen gels CL-PC

Cells used in these 3D-models

- Cell lines like LNCaP, PC-3, Du-145

- primary cells - NEC/CEC
  - normal prostate tissue
  - radical prostatectomy
  - palliative TUR

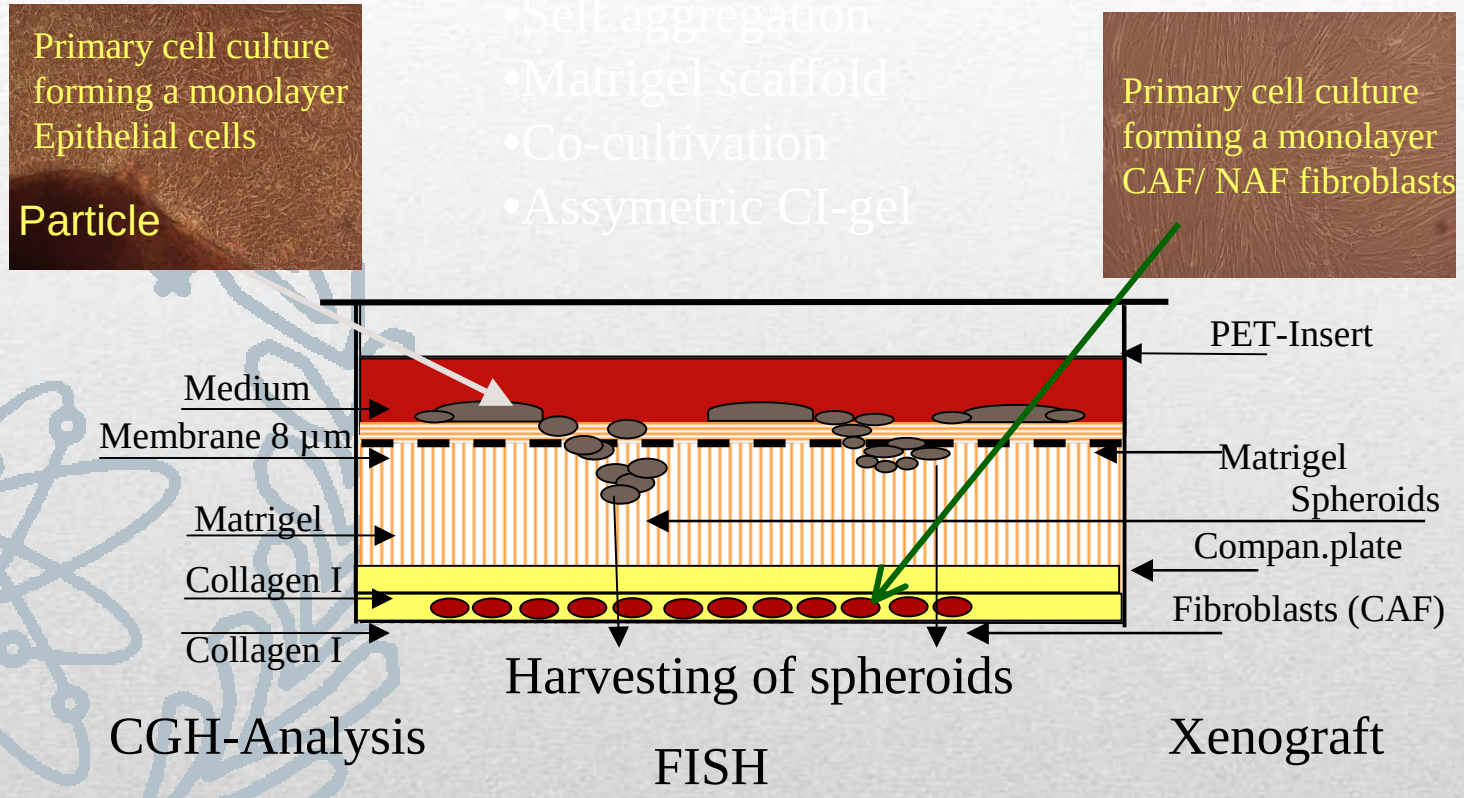
- primary cells – fibroblasts
  - normal fibroblasts (NAF)
  - myofibroblasts
  - cancer associated fibroblasts (CAF)

Modified by Saar A. Urologe A. 2011 Aug;50(8):961-7. doi: 10.1007/s00120-011-2630-7. German.

One promising approach

Hornburger 3D-Tumor-Invasion-Model

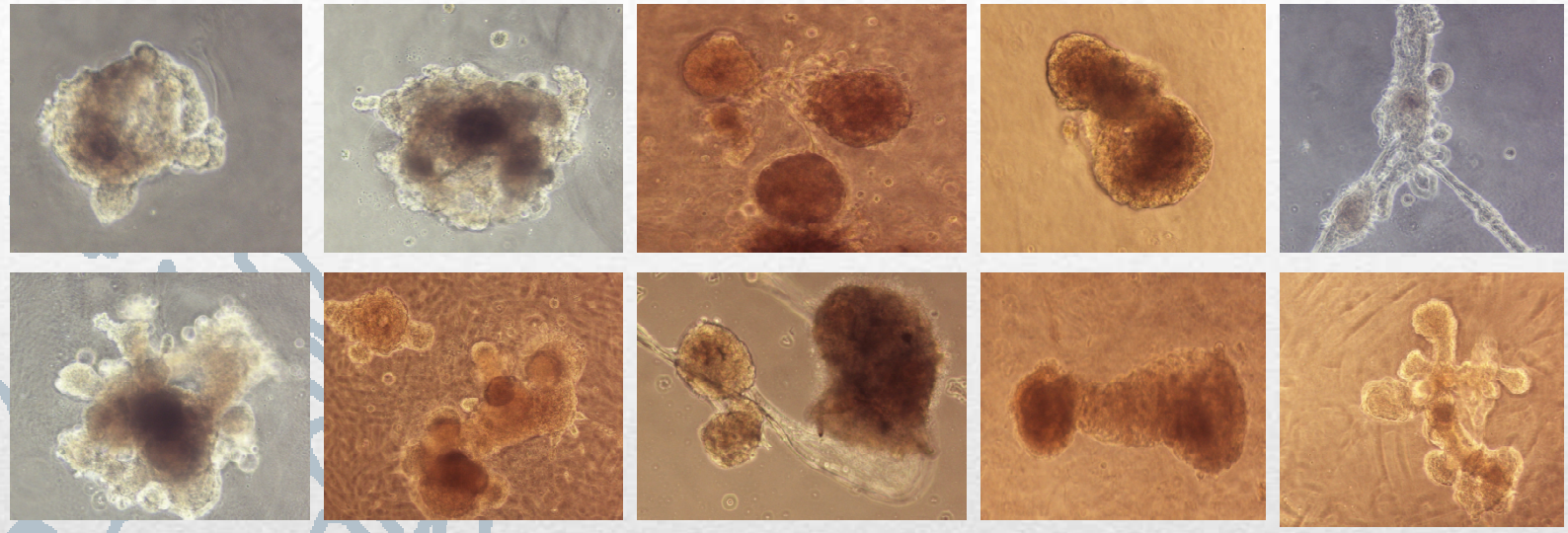
Combination of:
- Self aggregation
- Matrigel scaffold
- Co-cultivation
- Assymetric CI-gel



CAVE: CAF only as attractant not involved in spheroids

Modified by Saar A. Urologe A. 2011 Aug;50(8):961-7. doi: 10.1007/s00120-011-2630-7. German.

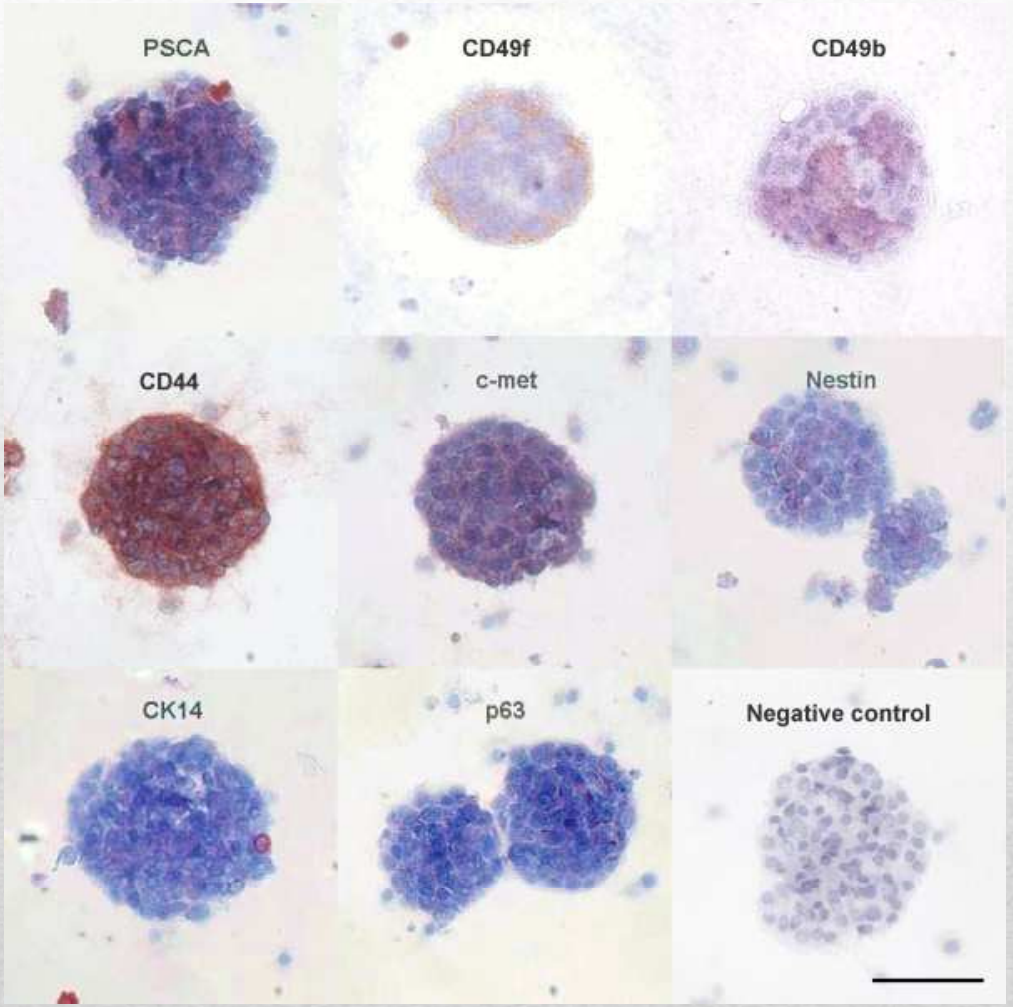
3D-Spheroids after invasion



Spheroids offer remarkable diversification in morphology

All invasive 3D-spheres show *uniform genetic gain/loss*
Non-invasive growing tumor cells exhibit *no genetic alterations*

3D-Spheroids after invasion



The Prostate 69:1683 – 1693 (2009)

Summary

Genome Landscapes and Tumorigenesis

- Clonal evolution and stochastic model
- Cancer stem cells model
- Cancer Risk Factors: genetic suscettibility
- Inflammation and cancer

Signalling Pathways in Human Cancer

- Oncogenic driver and tumour suppressor
- Oncogenes induces proliferation target proteins
- Hormone dependent pathways/refractory tumors

Genome editing and personalized medicine

- Baculoviruses as vectors for gene therapy against Human Cancer
- Cancer immunotherapy: Macrophages as cellular delivery
- Hormonal therapy, chemotherapy radiotherapy and surgery

Study Methods: new approach

Future Perspectives and Conclusions

Future perspectives

The information from cancer genome studies can also be exploited to improve methods for prevention and early detection of cancer, which will be essential to reduce cancer morbidity and mortality.

cDNA and miRNA can be used as prognostic and predictive factors of response to therapy in patients. CTCs and miRNA as prognostic factors.

- Circulating Tumor Cells recruited by the "liquid biopsy" might in the future substitute the “solid biopsy” that needs more invasive exams.
- It is an EpCAM-based method for isolation of CTCs in blood of cancer patients

Conclusions (I)

- Most human cancers are caused by two to eight sequential alterations that develop over the course of 20 to 30 years

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- Each of these alterations directly or indirectly increases the ratio of cell birth to cell death.

- The evidence to date suggests that there are ~140 genes whose intragenic mutations contribute to cancer but the definitive identification of these genes has been challenging.

- The known driver genes function through a dozen signaling pathways that regulate three core cellular processes: cell fate determination, cell survival, and genome maintenance.

Conclusions (II)

- Every individual tumor, even of the same histopathologic subtype as another tumor, is distinct with respect to its genetic alterations, but the pathways affected in different tumors are similar.
- Genetic heterogeneity among the cells of an individual tumor always exists and can impact the response to therapeutics.
- In the future, the most appropriate management plan for a patient with cancer will be informed by an assessment of the components of the patient's germline genome and the genome of his or her tumor.
- The information from cancer genome studies can also be exploited to improve methods for prevention and early detection of cancer, which will be essential to reduce cancer morbidity and mortal
- Genome Landascapes

Acknoledgment

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