JOINT MEETING
1ST ADVANCED AIRB COURSE IN RADIOBIOLOGY
BRESCIA MEETINGS IN RADIATION ONCOLOGY - 2015 EDITION

THE POWER OF BIOLOGY

Brescia – October 8th/9th, 2015

I TESSUTI:

1. Repair, Radiosensitivity, Recruitment, Repopulation, Reoxygenation
2. Acute and chronic hypoxia
3. Tissue microenvironment and tissue organization

Dott.ssa Liliana Belgioia
Università degli Studi di Genova

The 5 R's of radiobiology are the concept to explain the rationale behind the fractionation of radiotherapy

1975 Withers -> 4 Rs of radiobiology
(repair, recruitment, repopulation,reoxygenation)

1989 Steel -> fifth R
radiosensitivity

Repair:

Restoration of the integrity of damaged macromolecules

Recovery:

Increase in cell survival or reduction in the extent of radiation damage to a tissue, when time is allowed for this to occur.

Radiation damage:

1. Lethal damage -> irreversible and irreparable and cause cell death
2. Sublethal damage (SLD) -> can be repair in hours unless additional sublethal damage is added with which it can interact to form lethal damage
3. Potentially lethal damage (PLD) -> can be modified by post irradiation environmental conditions

DNA repair pathways:

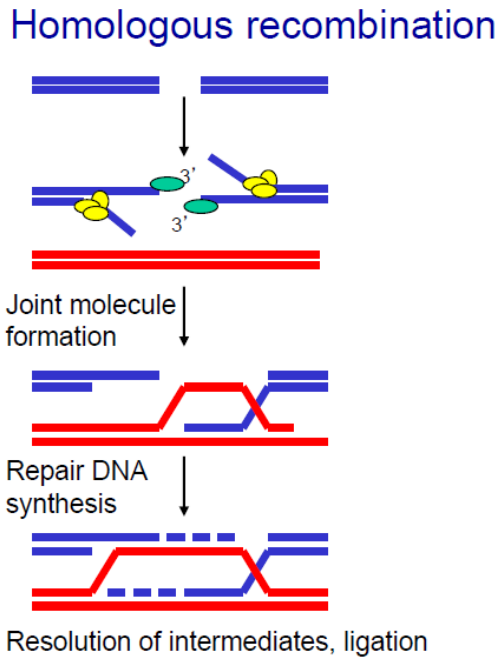
- Nucleotide Excision Repair
- Base Excision Repair
- DNA double strand break repair:
  - Homologus recombination (HR)
  - Non homologous end joining (NHEJ)

Homologous recombination:

- Requires homologous undamaged DNA strand
- Same base sequence
- Error free
- Several hours

When?

- S/G2 phase
- Only in division cells

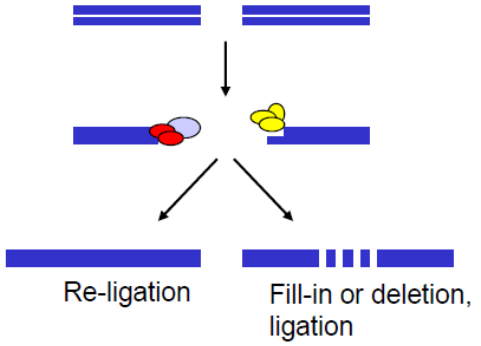


Non- homologous end-joining:

- Joins 2 DNA DSB ends together without homologous DNA sequences
- Rapid but less accurate
- Small deletion or insertions at the rapaired break site
- Maximize change of survival

- All phases of cell cycle
- All cells and tissues

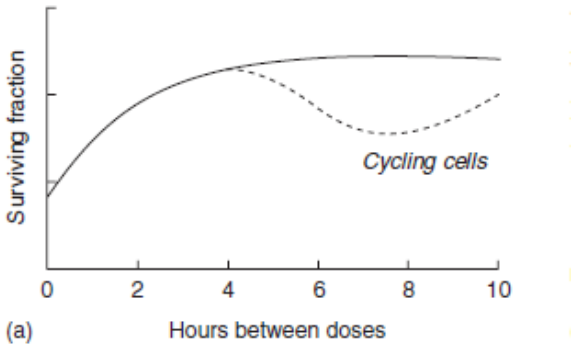
Non-homologous end-joining



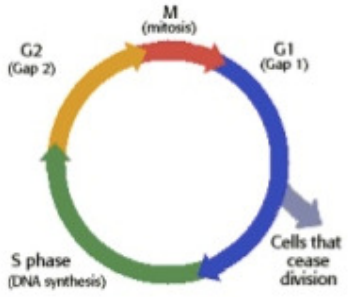
Split-dose experiments

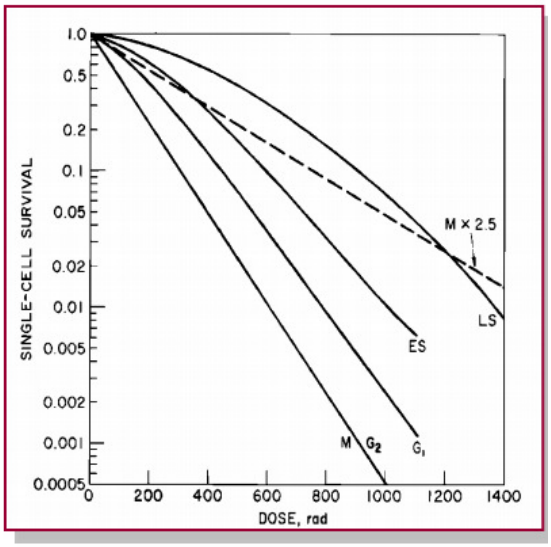
- Effect of dose of radiation is less if it is split in 2 fx, delivered a few hours apart -> recovery from sublethal damage
- Different endpoints: cell survival, tumour growth delay, mouse lethality after RT

Timing:
- Occurs 15 min- 1 h
- Completed (?) ~ 6 h
- But slower in some normal tissue (spinal cord)



Redistribution:





Redistribution:

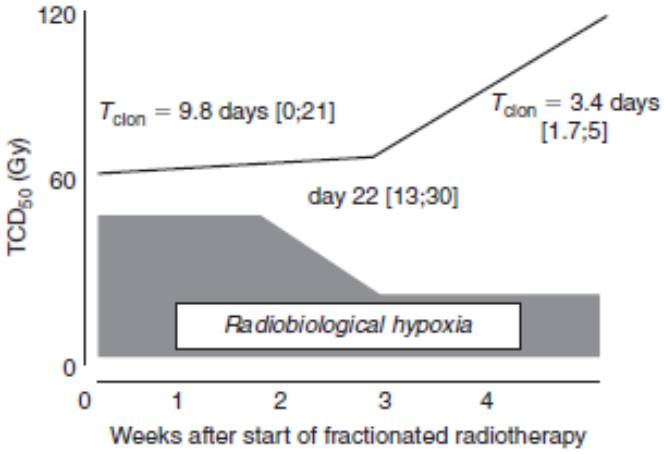
Synchrony in cells that survive irradiation -> all cells will be at the same point in cell cycle as before RT but greatest in S phase

with increasing time after RT-> cells will have the same distribution in cell cycle as before RT

Repopulation:

- Describes the proliferation of surviving clonogenic tumour cells during fractionated radiotherapy.

↳ Rapid repopulation during RT -> treatment resistance



Repopulation

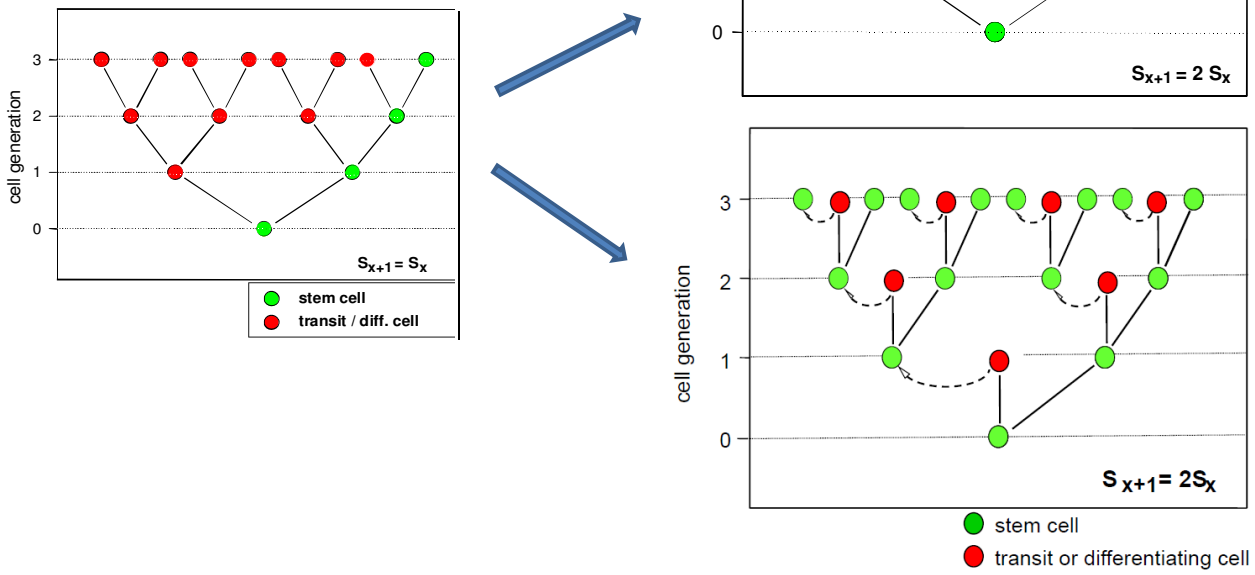
Also describes the regeneration response of early-reacting tissues to fractionated irradiation, which results in an increase in radiation tolerance with increasing overall treatment time.

Mechanisms:

- Asymmetry loss
- Acceleration
- Abortive divisions

Asymmetry loss

Asymmetrical division



Acceleration:

- Average cell cicle time: 3,5 days -> 1,4 days
- Cell cicle times shorter if asymmetry loss is incomplete or if higher doses are compesated

Abortive divisions:

Cell number near constant

Differentiation and cell loss continue

Cells production

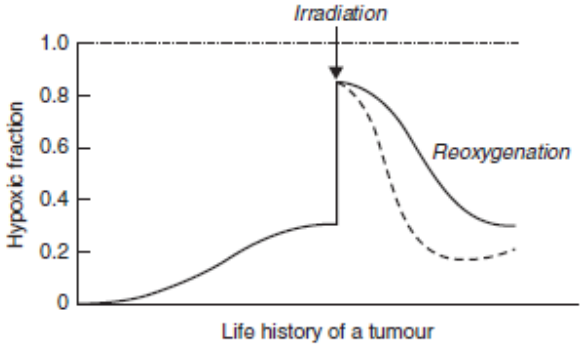
Sterilized cells (limited proliferation)

Stem cell ???

	Mechanism
Dose compensation	Asymmetry loss
Rate of dose compensation	Acceleration
Compensation of cell loss	Abortive division

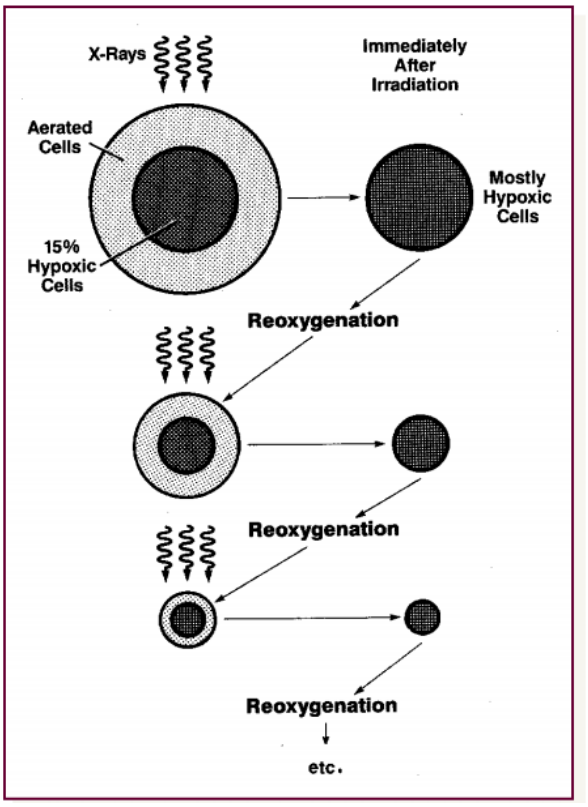
Reoxygenation:

The processes by which surviving hypoxic clonogenic cells become better oxygenated during the period after irradiation of a tumour.

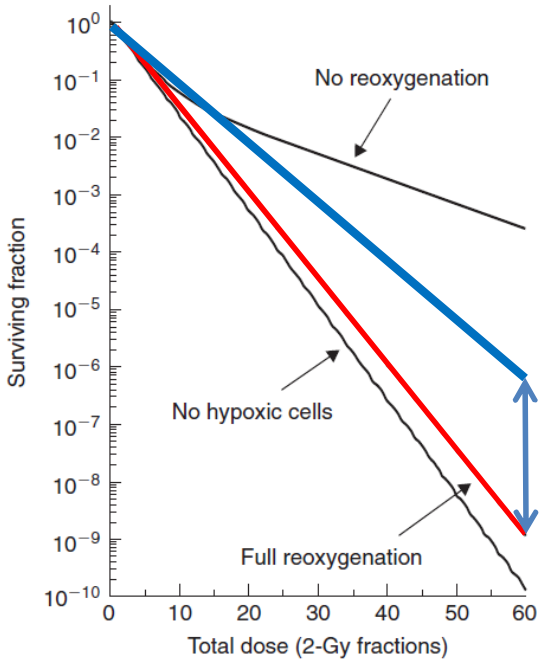


- reported in a variety of tumour systems
- the speed of reoxygenation varies widely - few hours several days

- Tumors contain a mixture of oxic and hypoxic cells
- A dose of x-rays kills a greater proportion of the oxic cells as they are more radiosensitive (OER)
- Immediately after RT most cells in tumors are hypoxic
- However pre-irradiation patterns tend to return because of reoxygenation
- Fractionation tends to overcome hypoxia



- Hypoxic cells are less sensitive to radiation
- Important cause of treatment failure
- Reoxygenation has been shown to occur in animal tumors
- Cells at intermediate levels of oxygenation
- Significative radioresistance



Wounters and Brown, 1997

The mechanisms underlying reoxygenation in tumours are not fully understood.

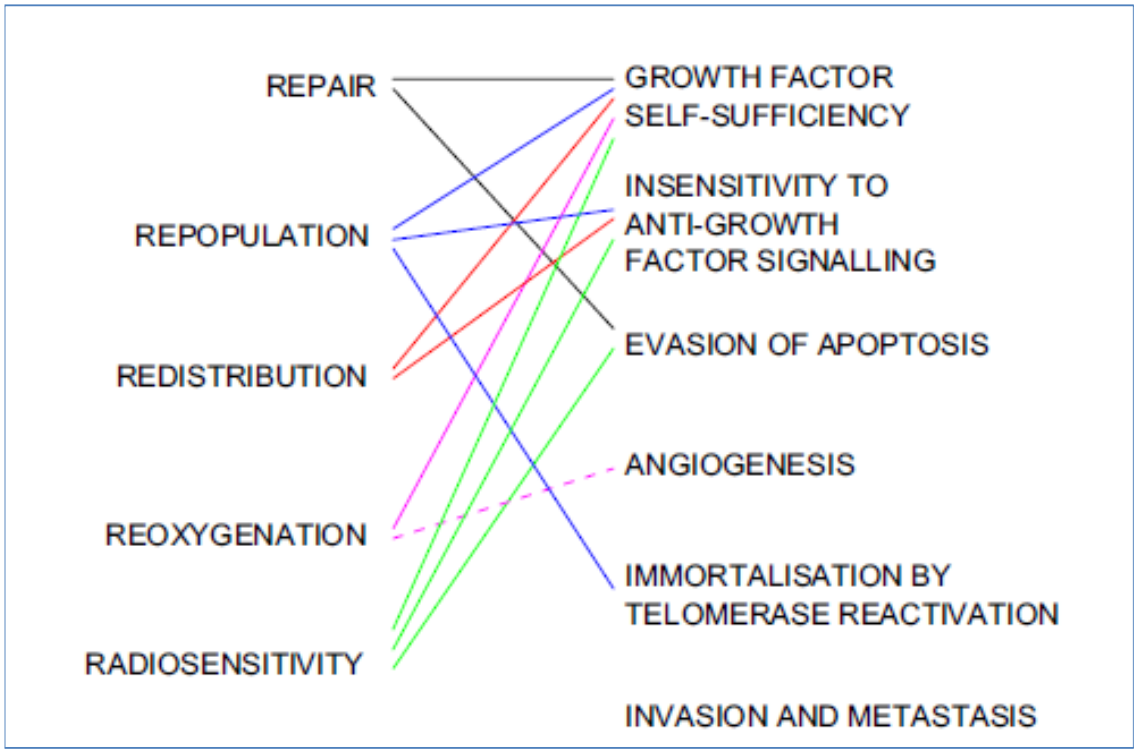
Mechanism	Time
Recirculation through temporarily closed vessels	Minutes
Reduced respiration rate in damaged cells	Minutes to hours
Ischaemic death of cells without replacement	Hours
Mitotic death of irradiated cells	Hours
Cord shrinkage as dead cells are resorbed	Days

Radiosensitivity:

There is an intrinsic radiosensitivity in different cell types.

Increasing sensitivity to Radiation:

- Lymphocites
- Erythrocytes, Granulocytes
- Epithelial cells
- Endothelial cells
- Connective tissue Cells
- Bone Cells
- Nerve Cells
- Brain Cells
- Muscle Cells



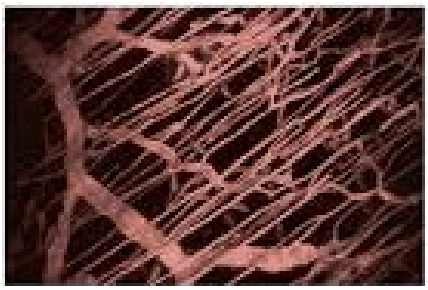
Harrington K. Clin Oncol 2007

1. Repair, Radiosesitivity, Recruitment, Repopulation, Reoxygenation

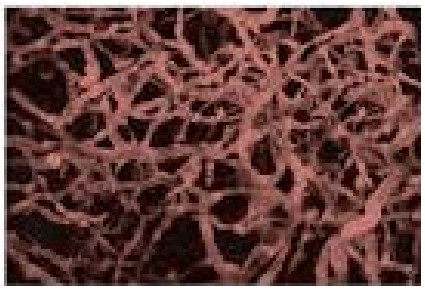
2. Acute and chronic hypoxia

3. Tissue microenvironment and tissue organization

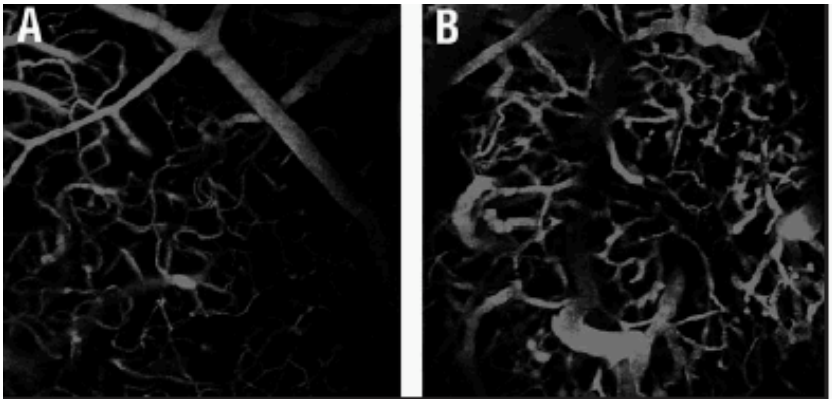
ABNORMAL VASCOLARIZATION



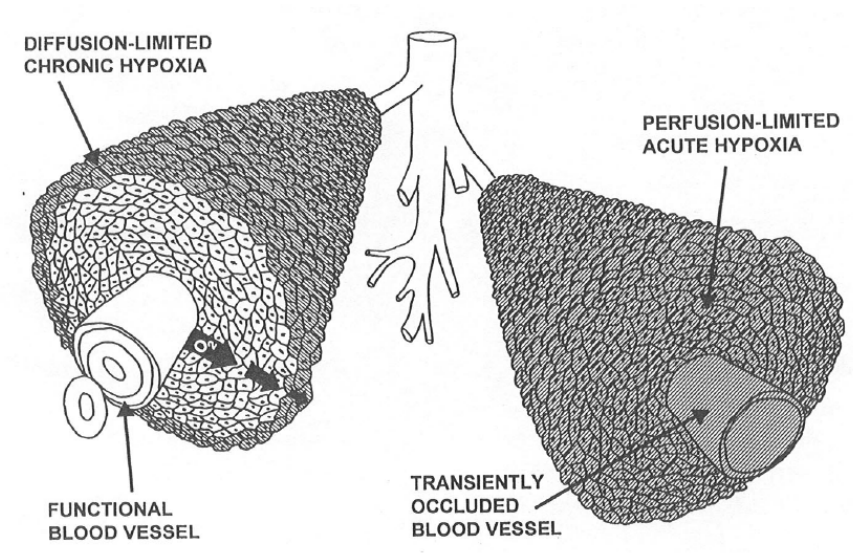
normal tissue



cancer



Acute and chronic hypoxia



1955 Thomlinson and Gray: chronic hypoxia
1979 Brown: acute hypoxia

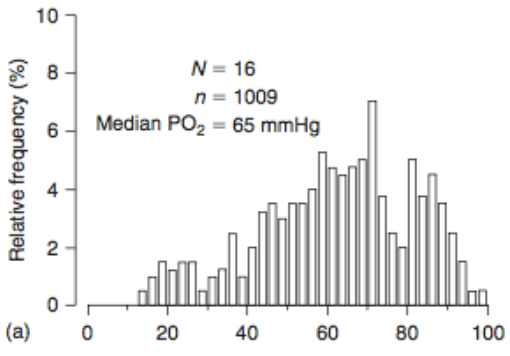
Hypoxia Heterogeneity

- In severity (Oxygen concentration)
- In space
- In time
- Amongst patients

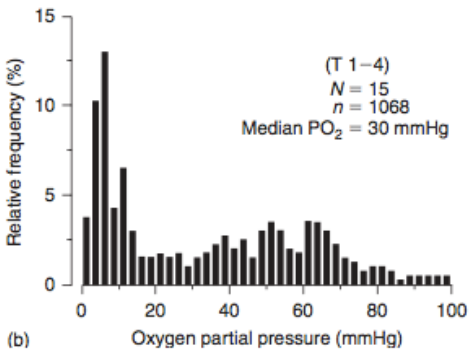
Heterogeneity in " severity"

Tumour cells at widely different oxygen levels <-> limitation in diffusion and perfusion

- Chronic hypoxia -> cell exist from normoxic to anoxic
- Acute hypoxia -> complete or partial limitation in perfusion

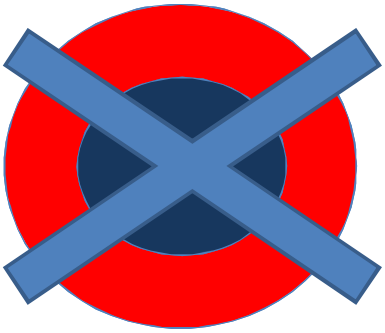


Breast tissue

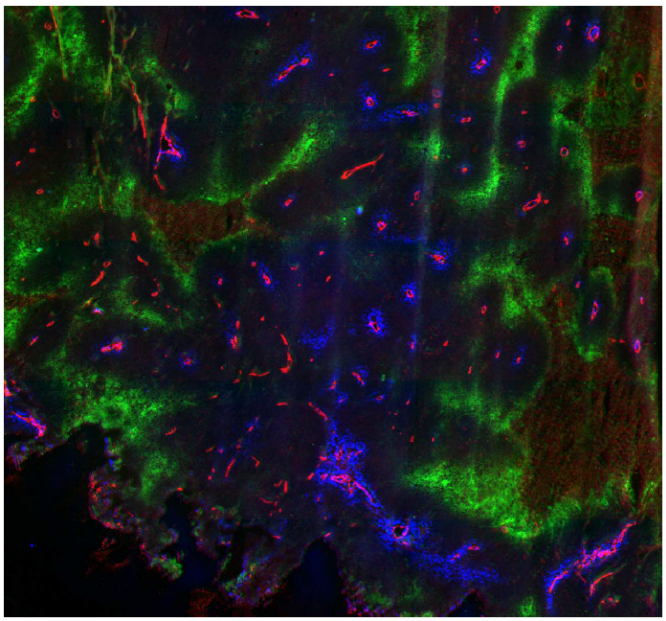


Breast cancer

Heterogeneity in "space "



- Hypoxia exists around every blood vessels, no association with tumour size
- Oxygenation varies at cellular level



Estro course, Bruxelles 2015

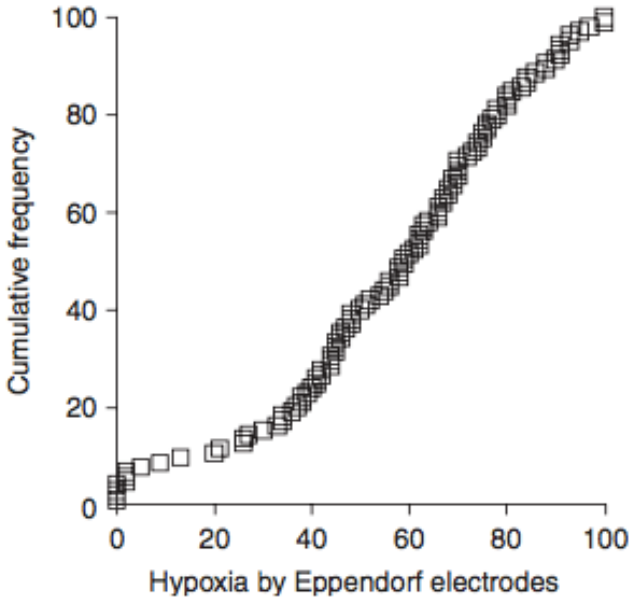
Heterogeneity in " time "

- Biological consequences -> time exposed to hypoxia
- Chronic Hypoxia ->
  - Cellular proliferation -> cells ↓ O2 over time as pushed away from vessels
  - Rate (exposure time) -> rate of proliferation that vary from one tumour to another and within different region of the same tumour -> hours to days
- Acute hypoxia -> linked to more rapid and dramatic O2 fluctuation

- Substantial proportion of tumour cells experience transient period of hypoxia lasting less than 1 hour

Heterogeneity among patients:

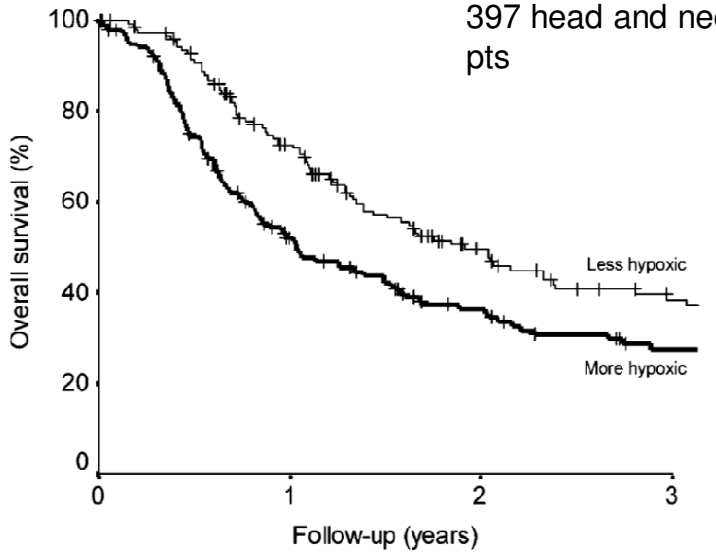
- Greater variability between different tumours
- Tumour with similar characteristic can display very different patterns
- 105 pts with cervical cancer



Nordsmark et al, 2006

Hypoxia as prognostic factor:

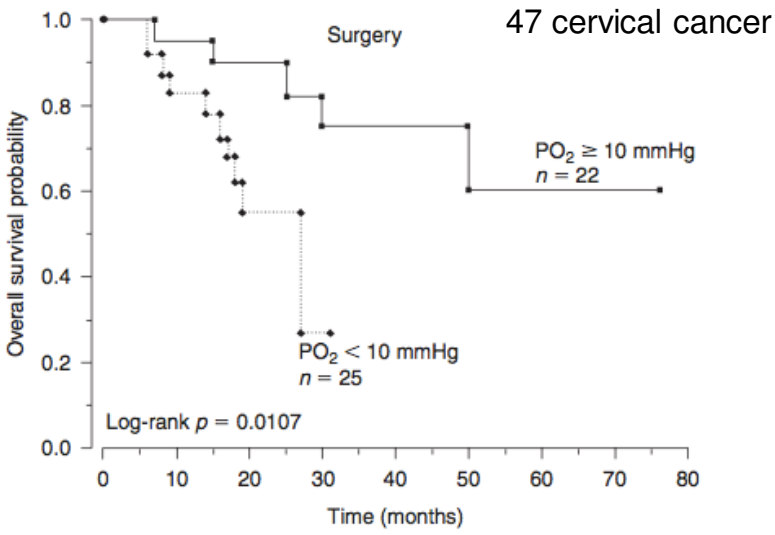
397 head and neck pts



O2 can be used to divide pts into different prognostic group -> different tp

Nordsmark et al, 2005

Hypoxia as prognostic factor:



- Hypoxia role unrelated to treatment sensitivity
- Hypoxic tumours biologically different from well oxygenated tumour
- Hypoxia strong predictor of M+

Hoeckel et al 1996

- Hypoxia influences malignancy:

1. adaptation
2. selection of malignant cells
3. promote genomic instability

Adaptation:

- Normal cells -> physiological pathways to adapted to low O2:

- High altitude -> ↑ EPO
- Heavy exercise -> anaerobic metabolism

- Hypoxia -> powerful regulator of angiogenesis

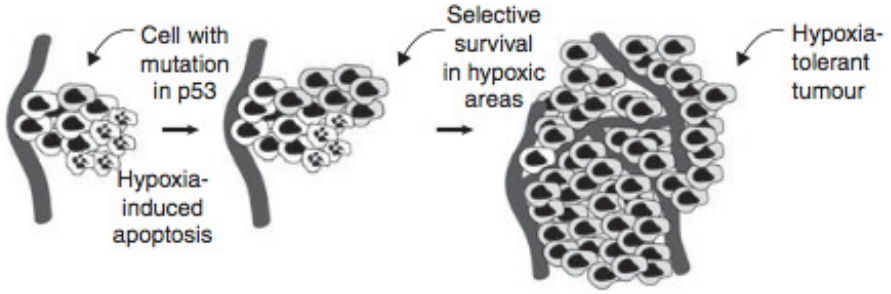
- Cancer-> same mechanisms

- Switch to glycolisis
- Stimulate angiogenesis

But genetic alterations -> physiological responce to hypoxia even in aerobic conditions

Selection of malignant cells

- Prolonged hypoxia might be toxic to tumour cells
- High tolerant
- Mutation in genes that regulate apoptosis



Conclusions:

- Two hypoxia mechanisms
- Hypoxia is eterogeneus
- Hypoxia can promote malignancy

1. Repair, Radiosensitivity, Recruitment, Repopulation, Reoxygenation
2. Acute and chronic hypoxia
3. Tissue microenvironment and tissue organization

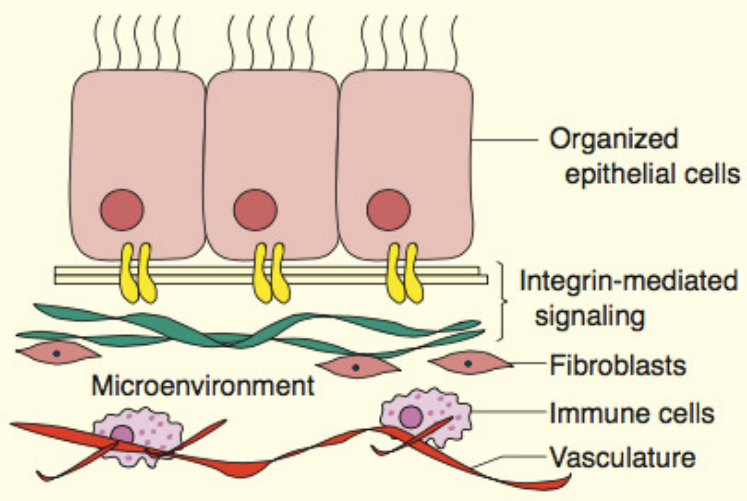
Tissue microenvironment and organization

Cancer as cell-autonomus process -> mutations in oncogenes and tumour supressors linked to cell proliferation

BUT

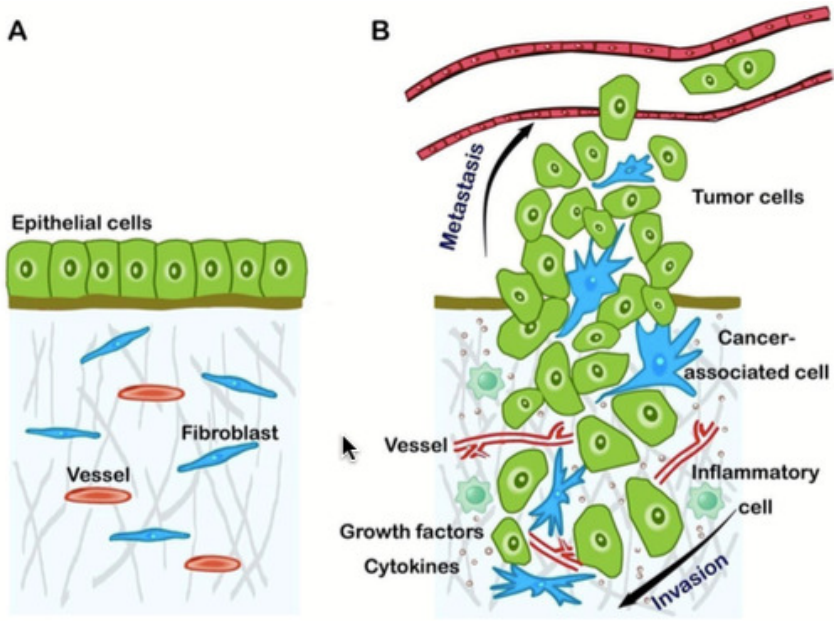
Tumour genesis and progression are determined also by favorable tumour microenvironment (TME)

TME



TME:
1- Stromal tissue
2- Insoluble extracellular matrix (ECM)
3- Milieu of cytokines and growth factors

<u>Basement membrane:</u>
Highly organized ECM but composed differently from stromal ECM to which epithelial cells attach



- Tumour as a complicated “organ”
- Highly eteregeneous population of cells

Stromal tissue

It is the supportive and connective tissue of the host tissue, it is composed by different cells as

- Fibroblasts
- Vasculature and lymphovasculatur endothelial cells
- Resident immune cells

There is a bidirectional, dynamic very intricate interaction between the cells of tumour stroma and cancer cells

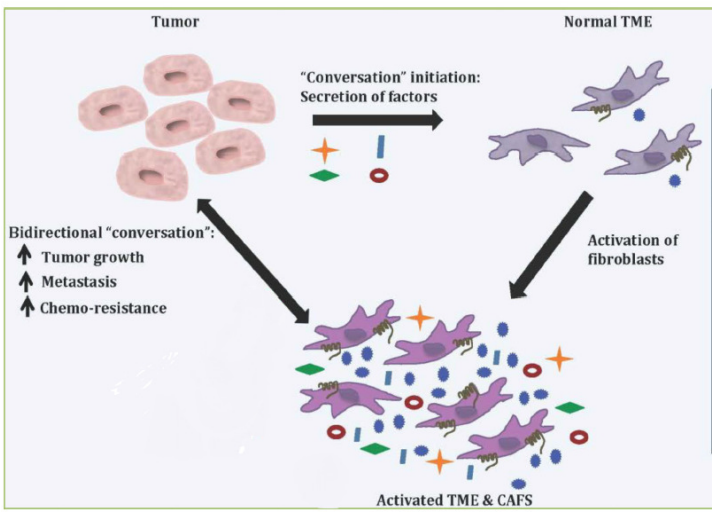
Stromal tissue - Fibroblast

- Fibroblast ->principal cellular component
- Under normal condition -> inactive quiescent state and synthesized components of ECM as collagens, laminin and fibronectin
- When activated - called CAFs (cancer associated fibroblasts) - higher proliferative activity, persinstently exist in tumour milieu and cannot be removed by apoptosis
- CAFs heterogeneous cell group -> several source

Stromal tissue - Fibroblast

CAFs play an integral role in tumour stromal interaction in several ways:

1. contribute to tumour cell growth and invasion -> ↑ production of ECM proteins
2. suppress immune responce
3. promote tumour angiogenesis and metastasis



Extracellular matrix (ECM)

- It is composed of collagen, elastin, proteoglycans and other structural proteins that provide support to and dividion of the cells of the host tissue
- These components make up both basement membrane and interstistial matrix
- ECM components confers unique physical,biochemical and biomechanical properties that are essential for regulating cell behaviour:

- properties are not indipendent
- ECM is highly dynamic
- cell-ECM interactions are reciprocal

Extracellular matrix (ECM)

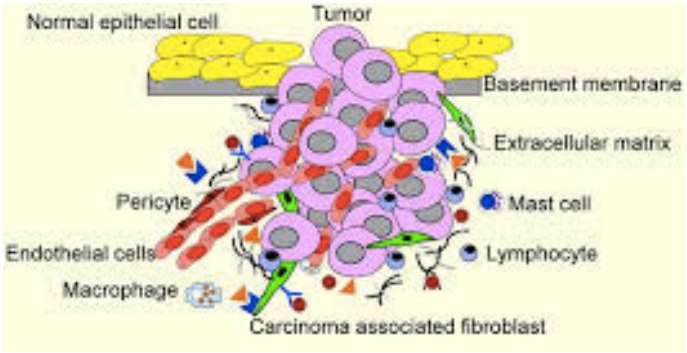
- Tumours progression requires a continually elvolving network of interactions between neoplastic cell and ECM -> first step is remodelling of ECM
- Many ECM proteins are synthetized by fibroblast -> in cancer CAFs secrete proteins that degrade ECM
- ECM is remodelling by matrix metalloproteinases (MMPs)

MMPs are pro angiogenetic and metastatic

1. the digestion of ECM by proteases allow the entry of cancer cells into the host tissue allow the entry of the cancer cells through the barrier of the host tissue

2. migration of endothelial cells into the matrix that results in neovascularization

3. cause a release of growth factors -> amplify tumour growth and invasion



Close link between tumour cells and TME:
- Basement membrane is degraded
- ECM is overproduced

Epithelial to mesenchymal transition
Activation fibroblasts and inflammatory cells
Newly formed vasculature

Alter and facilitate tumour progression and metastasis

Conclusions:

- Neoplastic cells are influenced by the surrounding microenvironment and viceversa
- TME is an integral part of the tumour that not only provide tumour architectural support but also affects its physiology and function
- Manipolation of interaction between tumour cells and TME -> novel therapeutic approach