Pathobiochemistry of tumorogenesis

Pathobiochemistry of malignant transformation

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Malignant cancer – the genetic disease

Cancer intro:

Affection of genomic DNA by various pathogenetic events

Normal tissue

Malignant transformation

Malignant tumor formation
Affection of genomic DNA by various pathogenetic events

Cancer intro:

Malignant cancer – the genetic disease

Malignant transformation

Normal tissue

WHICH GENES?

WHICH PROCESSES?

WHICH CELLS?

Malignant tumor formation
A few introductory remarks:
- cells in multicellular organism
- known facts about malignant transformation
- cell renewal
Growth control in multicellular organism: 

*Considered* **hierarchical building plan**

- **Organism** → **Organs** → **Tissues** → **Tissue clusters** → **Cells**
- $1 \rightarrow 10^2 \rightarrow 10^3 \rightarrow 10^8 \rightarrow 10^{14}$ [entities]

$10^7 \times$ population
Growth control in multicellular organism:

Regulated by hierarchical chemical signalling

- Hormones
- Local signals
  - Growth factors
  - Cytokines
  - Apoptotic regulators
  - Etc.

Organism → Organs → Tissues → Tissue clusters → Cells
Growth control in multicellular organism:

Regulated by hierarchical chemical signalling

- **Organism** → **Organs** → **Tissues** → **Tissue clusters** → **Cells**

- **Hormones**
- **Local signals**

**Surveillance by continuous control**

- Self?
- Proper?
- Controlled?
Growth control in multicellular organism:

Regulated by chemical signalling at the cellular level

Considering the intracellular conditions and local environment in a tissue cluster
Growth control in multicellular organism:

Regulated by *chemical signalling* at the cellular level

Considering the intracellular conditions and local environment in tissue cluster

Cells (not the organism) autonomously regulate the absolute amount of entities in a tissue clusters
Cellular growth control:

*Interactions of local and distant factors*

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Cellular growth control:

Represented by *biochemical interactions*

| chemical signalling & intracellular conditions & local environment in tissue cluster |
|-------------------------------------------------|-----------------------------------|-----------------------------------------------|
| • Hormones                                     | • Cell integrity                        | • Resources                                  |
| • Local signals                                | • DNA integrity                         | • Space, contacts                            |
| • „Immune“ signals                             | • Energetics                             | • Stromal interaction                        |
| • signal ligands                               | • cell membrane                         | • nutrients                                  |
| • receptors                                    | • mitochondrion                         | • oxygen                                     |
| • signal transducers                           | • damage sensors                        | • integrins                                  |
| • IC signaling networks                        | • transducers                           | • cell-cell interaction                      |
| • transcription factors                        | • DNA repair                             | • molecules                                  |
| • executive proteins                           | • telomere                               |                                              |
|                                                | • maintenance                           |                                              |
|                                                | • ATP production                        |                                              |
A few introductory remarks:
- cells in multicellular organism
- known facts about malignant transformation
- cell renewal
Cellular growth control:

Represented by biochemical interactions **regulating**

- **chemical signalling**
  - Hormones
  - Local signals
  - „Immune“ signals

- **intracellular conditions**
  - Cell integrity
  - DNA integrity
  - Energetics

- **local environment in tissue cluster**
  - Resources
  - Space, contacts
  - Stromal interaction

- **Cell replication**
  - Signal ligands
  - Receptors
  - Signal transducers
  - IC signalling networks
  - Transcription factors
  - Executive proteins

- **Cell death**
  - Cell membrane
  - Mitochondrion
  - Damage sensors
  - Transducers
  - DNA repair
  - Telomere maintenance
  - ATP production

- **Differentiation**
  - Nutrients
  - Oxygen
  - Integrins
  - Cell-cell interaction molecules

- **Senescence**
  - Nutrients
  - Oxygen
  - Integrins
  - Cell-cell interaction molecules

- **Migration**
  - Nutrients
  - Oxygen
  - Integrins
  - Cell-cell interaction molecules
Cellular growth control: Physiologically - balanced state in tissues

Tissue homeostasis

- Cell replication
- Cell death
- Differentiation
- Senescence
- Migration
Cellular growth control:

Critical events for tissue homeostasis maintenance

- Cell replication
- Cell death
Cellular growth control:

Critical events for tissue homeostasis maintenance

Cellular

Regression → Progression

Cell replication
- Mitotic signaling pathways
- Cell cycle regulation

Cell death
- Apoptotic pathways
Cellular growth control:

**Critical events** for tissue homeostasis maintenance

- **Cell replication**
- **Cell death**

**Critical conditions:**
- healthy (intact) DNA
- competent immunity system
Cellular growth control:

**Critical events** for tissue homeostasis maintenance

Critical conditions:
- **healthy (intact) DNA**
- **competent immunity system**
  - DNA repair mechanisms
  - Cell cycle checkpoints
  - Differentiation
Cellular growth control:

Pathologically – deterioration of tissue homeostasis

Tissue homeostasis

Cell replication

Migration

Cell death

Senescence

Differentiation
**Cancer:**

Specific *deterioration of tissue homeostasis*

Cell replication

↑ Mitotic signaling pathways  ↓ Apoptotic pathways

↑ Cell cycle regulation
Cancer: Specific deterioration of tissue homeostasis

Enabled by:
- Damaged DNA
- Uncompetent immunity system

- DNA repair mechanisms
- Cell cycle checkpoints
- Differentiation
Malignant transformation:

Specific deterioration of tissue homeostasis

| Mitotic hyperstimulation | ▪ Growth of malignant tissue  
|                          | ▪ Insensitivity to local and systemic growth control |
| Cell cycle upregulation  | ▪ Growth across the tissue architecture |
| Evasion of apoptosis      | ▪ Immortalization |
| Deterioration of DNA repair | ▪ Toleration of genetic alterations |
| Impaired differentiation  | ▪ Clonal variability |
                                                                 | ▪ Immature phenotype |
                                                                 | ▪ Metastatic potential |
                                                                 | ▪ Escape from immune surveillance |
A few introductory remarks:
- cells in multicellular organism
- known facts about malignant transformation
- cell renewal
Cell growth control in tissues:

**Majority of tissues have self renewal capacity**

provided by gradual maturation from tissue stem/progenitor cells
Cell growth control in tissues:

Majority of tissues have self renewal capacity

provided by gradual maturation from tissue stem/progenitor cells

Stimulated by many (?), mainly local, factors

[entities in hypothetical tissue]
Cell growth control in tissues:

Majority of tissues have self renewal capacity provided by gradual maturation from tissue stem/progenitor cells

- **Supplementation of progenitors**
  - Divisions: unlimited
  - Mitotic activity: low
  - Phenotype: unmaturated

[entities in hypothetical tissue]
Cell growth control in tissues:

**Majority of tissues have self renewal capacity**

provided by gradual maturation from tissue stem/progenitor cells

- **Supplementation of progenitors**
  - Divisions: unlimited
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  - Phenotype: unmaturated

- **Supplement desired cell types**
  - Divisions: limited (100x)
  - Mitotic activity: high
  - Phenotype: partially maturated

(entities in hypothetical tissue)
Cell growth control in tissues:

**Majority of tissues have self renewal capacity**

provided by gradual maturation from tissue stem/progenitor cells

1. **Stem cell**
   - Supplementation of progenitors
   - Divisions: unlimited
   - Mitotic activity: low
   - Phenotype: unmaturated

2. **Progenitor 1**
   - Supplementation desired cell types
   - Divisions: limited (100x)
   - Mitotic activity: high
   - Phenotype: partially maturated

3. **Progenitor 2**
   - Providing specific tissue functions
   - Divisions: very limited (10x)
   - Mitotic activity: variable
   - Phenotype: maturated

[entities in hypothetical tissue]
Origin of cancer cells:

Genetic aberrations in stem / progenitor cells provide propagation of cancer-prone mutations to downstream cells.

- **Supplementation of progenitors**
  - Divisions: unlimited
  - Mitotic activity: low
  - Phenotype: unmaturated

- **Supplement desired cell types**
  - Divisions: limited (100x)
  - Mitotic activity: high
  - Phenotype: partially maturated

- **Providing specific tissue functions**
  - Divisions: very limited (10x)
  - Mitotic activity: variable
  - Phenotype: maturated
Examples of:

- mitotic hyperstimulation
- defects of DNA repair mechanisms
- evasion of apoptosis
Signals of cascades pathways for mitotic stimulation

- Growth factors, cytokines, and lipophilic hormones
- Their receptors
- Signal transducers
- Intracytoplasmic kinases
- Nuclear receptors and transcription factors
- Cell cycle regulators

Mitotic signal

- Signal recognition and its transport across cell membrane
- Transmission of mitogenic signal from receptors to intracellular transducers
- Amplification and propagation of the signal toward the cell nucleus
- Transmission of mitotic signals to the nucleus

DNA: Gene expression changes
Signals of cascades pathways for mitotic stimulation

- Growth factors
- Cytokines
- Their receptors

Signal transducers
- Intracytoplasmic kinases

Intracytoplasmic kinases
- PI3K
- PDK1
- Akt/PKB
- Raf
- MEK
- mTOR
- ERK

Nuclear receptors and transcription factors
- Transcription factors
- STAT
- DNA:

Cell cycle regulators
- Expression of cell cycle regulators
Signaling of receptor tyrosine kinases

- SH2 domain
- Farnesyl transferase
- Palmitoyl transferase
- Ras GDP
- P
- GTP
- GDP
- Pi
- GEF
- GAP
- Sos
- Grb
- MAPK cascade
- PI3K
- AKT/PKB
- Transcription regulation
- Proliferation
- Apoptosis
Defects of receptor tyrosine kinases signaling

Hyperproduction of GF and/or their receptors

- Many cancer cells overexpress production of growth factors
- ErbB-2 (EGFR member) overexpressed (by gene amplification) in ~25% breast cancers
- ErbB-2 (her2/neu) could be targeted by specific Ig (Herceptin)

MAPKK: MAPKK: MAPK:

MAPKK: MAPKK:

MAPK:

Sos Ras Grb GTP GTP PKC PI3K

Raf MEK ERK AKT/PKB

nucleus

Transcription regulation (eg. Ets-2, Elk-1) Apoptosis

Proliferation
Defects of receptor tyrosine kinases signaling

- Mutations in transducer
  - Mutation in \( k\text{-ras} \) oncogene: one of the most frequent event in solid cancers (~30%).
  - Mostly pancreatic (~80%) and lung (~40%) cancers.
  - Triggering the downstream pathways independent on presence of upstream stimulatory events.

MAPKKK:

MAPKK:

MAPK:

- Ras
  - GTP
  - PI3K

- Raf
  - PKC
  - AKT/PKB

- MEK

- ERK

- nucleus

- Transcription regulation (eg. Ets-2, Elk-1)

- Apoptosis

- Proliferation
Ras - directed therapy

Inhibitors of transferases

- Ras molecule fails to insert in submembranal space – defective signaling

Ras - GTP

Other pathways:
- Transcription regulation
- Proliferation
- Apoptosis

Pathways:
- PI3K
- AKT/PKB
- MAPK cascade

Transferases:
- Farnesyl transferase
- Palmitoyl transferase

Regulation factors:
- GAP
- GEF

Sh2 domain

Sos

Grb

P

SH2 domain

Ras

GTP

GDP

Pi
Defects of receptor tyrosine kinases signaling

Mutations in kinases

- Mutation in B-raf oncogene: melanoma (~70%), papillary thyroidal cancer (~50%) and colorectal (~40%) cancers.
- Specific therapy: antisense oligos and specific inhibitors of kinase activity

Transcription regulation (eg. Ets-2, Elk-1)

Proliferation

Apoptosis

MAPK: MAP KK: MAPKK: MAPKKK:

Ras GDP PI3K

PKC

Raf MEK ERK AKT/PKB

nucleus
PI3K signaling

Regulation of translation

Proliferation

mTOR

IKK

Caspase-9

Bad

FOXO

NFkB

PI3K [p85/p110]

PI2P

PI3P

PTEN

PDK1

Akt

GDP

Ras

Sos

Grb

PI3P

[pi85/p110]
Defects of PI3K signaling

Mutations/amplification in kinase

- PI3K [p58/p110]
- PTEN

Overexpression in kinase

- mTOR
- IKK
- Akt
- PDK1
- PI3P
- PI2P
- GDP
- Grb
- Ras
- Sos

- Mutations in PI3K (CA) – ovarian cancer
- Overexpression in AKT (PKB) – ER negative breast cancer
- Familial PTEN mutations – Cowden syndrome

Regulation of translation

Proliferation

Apoptosis

NFkB

Caspase-9

Bad

FOXO

CD95L/FasL

NFkB

IKK

mTOR

PDK1

PI3K

PI3P

PTEN

Grb

Ras

Sos

GDP

P

P

Mutations in phosphatase

- Mutations in PI3K (CA) – ovarian cancer
- Overexpression in AKT (PKB) – ER negative breast cancer
- Familial PTEN mutations – Cowden syndrome
Cytokine signaling canonical pathway

- Cytokine binding to cell surface receptor
- Adaptor proteins
- SOCS proteins
- MAPK cascade
- STAT proteins
- Gene expression regulation
Defects of cytokine signaling

- Mutations / amplification in kinase
- Down-regulation / hypermethylation of SOCS
- Overexpression of TFs
  - Upregulation of JAK/STAT pathways – hallmark of many lymphoproliferative diseases

Adaptor proteins

MAPK cascade

Gene expression regulation

SOCS

SH2 domain
Results of aberrant signaling pathways?

- Autonomous hyperstimulation of cellular growth potential
- Immaturity
- Enhanced migration potential
Cell cycle (CC): aims

Cell cycle – the sequence of consecutive biochemical events leading to cell replication

The aims of CC:

- Highly precise copying and even distribution of genetic material between the new daughter cells
- Creation of two identical daughter cells from mother cell
- Cell differentiation in some cases
Cell cycle: overview

Symmetrical gDNA and cell contents. Formation of daughter cells.

Restriction point. DNA complete or die!

S phase completion control. gDNA repairation. Preparing for mitosis.

Replication of gDNA. Synthesis of organelles.

Cell cycle entry. Preparing to gDNA replication.

Restriction point. Divide or die!

Terminal differentiation.
Cell cycle: overview

**Checkpoint / DNA repair failure**
- Restriction point.
- DNA complete or die!

**Mitotic hyperstimulation**
- Terminal differentiation
- Cell cycle entry.
- Preparing to gDNA replication
- Divide or die!

**G1**
- Replication of gDNA.
- Synthesis of organelles.

**G2**
- S phase completion control.
- gDNA reparation.
- Preparing for mitosis.

**M**
- Mitotic hyperstimulation
- Checkpoint / DNA repair failure

**G0**
- Restriction point.
- DNA complete or die!
Regulation of cell cycle entry

G1 phase:

S phase:
Regulation of cell cycle entry

G1 phase:

- Contact inhibition
- Rb
- E2F-1
- HDAC

S phase:

- Expression
- E2F-1
- HAT
- Rb (P, P, P)

Pathways:

- TGFβ -> SMAD -> p15, p16, p27
- ATM -> p53 -> p21
- Cdk4/6, Cyklin D
- Cdk2, Cyklin E
- E2F-1
- DNA damage

Key factors:

- Rb
- E2F-1
- HDAC
- SMAD
- p53
- TGFβ
- ATM
- Cdk4/6
- Cyklin D
- Cyklin E
- E2F-1
- HAT
- DNA damage
Perturbances of proper cell cycle entry

- **TGFβ** → **SMAD** → **p15, p16, p27**
- **Cyklin D** → **Cdk4/6**
- **Cyklin E** → **Cdk2**

**Contact inhibition**

- **ATM** → **p53** → **p21**

**Expression**

- **E2F-1**
- **HAT**

**Activation/amplification of oncogenes**

- **Inactivation/mutation of TSG**
- **Inactivation/mutation of CKI**

**G1 phase:**
- Contact inhibition
- Activation/amplification of oncogenes
- Inactivation/mutation of TSG

**S phase:**
- Expression
- Inactivation/mutation of TSG
Core DNA repair pathways (DSBR)

**NHEJ (G1/S phase)**
- DNA-PKcs
- PARP
- DNA-PKcs
- Ku70
- Ku80
- XRCC4
- LigaseIV
- Proteins of chromatin

**Homologous recombination (G2 phase)**
- ATM
- p53
- BRCA1
- BRCA2
- Rad51
- NBS1
- MRE11
- Rad17
- Rad1
- Rad9
- Rad26
- HUS1
- Proteins of chromatin
- PARP
- Poly ADP-ribose

**Cell cycle block**
- p53
- ATM
- Poly ADP-ribose
- Proteins of chromatin
Deterioration of DNA repair pathways (DSBR)

NHEJ (G1/S phase)

Homologous recombination (G2 phase)

Cell cycle block

Ataxia telangiectasia (AT)

Li-Fraumeni syndrome

Hereditary breast cancer

Inborn: Hereditary cancer syndromes

Somatic: Genomic instability

Proteins of chromatin

ATM

Poly ADP-ribose

BRCA1, BRCA2

RAD51

Proteins of chromatin

Poly ADP-ribose

PARP

Ku70, Ku80

DNA-PKcs

XRCC4, LigázaIV

MRE11, NBS1, Rad51

NBS1

Nijmegen breakage syndrome

AT-like

Hereditary breast cancer

P53

Hereditary breast cancer
Apoptosis pathways: overview

**Extrinsic pathway**
- Death receptors
- Caspases of proximal apoptotic pathway
- Executive caspases
- Substrates (enzymes, structural proteins, DNA)
- APOPTOSIS

**Intrinsic pathway**
- Mitochondrion
- Bcl-2 family
- Caspases of proximal apoptotic pathway
- Executive caspases
- Substrates (enzymes, structural proteins, DNA)
- APOPTOSIS
Apoptosis pathways: players
Apoptosis pathways: defects

Signal switch

Lost of activation signaling

Inhibition of caspase activation

Changes in Bcl-2 members

Inhibition of caspase activation

Substrates (Lamin, gelsolin, actin, ICAD)

Cytochrome C

APOPTOSIS
p53 – the most frequently mutated TSG

TP53 stably expressed under physiological conditions

Low IC concentration of p53 maintained by its targeting for ubiquitin mediated proteasomal degradation by mdm2 E3 activity
p53 – the most frequently mutated TSG

p53 – transcriptional regulation of CC & apoptosis following DNA damage

DNA damage (DSB)

ATM → p53 → mdm2

CHEK2

Bcl-2

Bax, PUMA

GADD45

G2 → M phase

Cell cycle

Cdc25

Cdk1

Cyclin B

Cdk1

APOPTOSIS
Malignant cancer – the genetic disease

Malignant transformation: summary

Normal tissue

malignant transformation

WHICH GENES?

WHICH PROCESSES?

WHICH CELLS?

Malignant tumor formation
Malignant cancer – the genetic disease

Malignant transformation: summary

Normal tissue

- Up-regulation of oncogenes (genes promoting cell cycle or inhibiting apoptosis)
- Downregulation / silencing of tumor suppressor genes (genes halting cell cycle or facilitating apoptosis)
- Inactivation of genes coding for DNA repair proteins maintaining DNA integrity

Which genes?

Which processes?

Which cells?

Malignant tumor formation
Malignant transformation: summary

**Malignant cancer – the genetic disease**

- Up-regulation of oncogenes (genes promoting cell cycle or inhibiting apoptosis)
- Downregulation / silencing of tumor suppressor genes (genes halting cell cycle or facilitating apoptosis)
- Inactivation of genes coding for DNA repair proteins maintaining DNA integrity

**Normal tissue**

**WHICH GENES?**

**WHICH PROCESSES?**

**WHICH CELLS?**

**WHICH MUTATIONS?**

**Malignant tumor formation**
Malignant transformation: summary

Malignant cancer – the genetic disease

- **Drivers**
  - mutations „driving“ malignant transformations

- **Passengers**
  - mutations that arise from disordered DNA repair processes that are NOT involved in tumorigenesis

**Normal tissue**

WHICH GENES?

WHICH PROCESSES?

WHICH MUTATIONS?

Malignant tumor formation
Malignant transformation: summary

Malignant cancer – the genetic disease

Normal tissue

- Activation of cell cycle (including the promitotic pathways)
- Evasion of apoptosis
- Impairment of DNA repair mechanisms
- Decreased maturation and senescence

Malignant tumor formation

WHICH GENES?

WHICH PROCESSES?

WHICH CELLS?
Malignant cancer – the genetic disease

Normal tissue

- Stem / progenitor cells rather than matured cells in tissues

Malignant tumor formation

- WHICH GENES?
- WHICH PROCESSES?
- WHICH CELLS?
Cancer pathways: Why we should know them?

- **Classification of tumors**
  - Identification of cancer genes and "driving" mutations

- **Improved, optimized treatment**
  - Targeting of tumor cells – not somatic cells
  - Individualization of therapy based on molecular cancer profile