BRAF and Malignant Melanoma

*From gene to cancer therapy*

Lezione di biotecnologie in Oncologia
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What is cancer?

- All cancers are genetic diseases
- All cancers derive from single cells that continually divide in an unrestrained manner
- Cancer cells behave in this abnormal way because of changes in the DNA sequence of key genes, which are known as cancer genes
- 1 in 3 people will develop cancer
- 1 in 8 people will die from cancer
- There are approximately 200 types of cancer, each with different causes, symptoms and treatments
- 309,527 people were newly diagnosed with cancer in the UK
- An individual's risk of developing cancer depends on many factors, including age, lifestyle and genetic make-up
What is a mutation?

- **Germline mutation**
  - A change in the DNA sequence that can be inherited from either parent

- **Somatic mutation**
  - A change in the DNA sequence in cells other than sperm or egg
  - The mutation is only present in the cancer cell and its offspring (not in the patient’s healthy cells)
Tumour suppressor gene

These genes normally function to SUPPRESS cell growth and division

Cancer
Proto-oncogene

Genes which normally function to PROMOTE cell growth and division in a controlled manner
Malignant melanoma

- Malignant melanoma originates in melanocytes, specialised pigment cells found in the skin.
- Melanoma accounts for 4-5% of all skin cancers but is responsible for 80% of deaths.
- New treatments are needed.
Melanoma progression

- **Stage**: Benign Nevus, Dysplastic Nevus, Radial-growth Phase, Vertical-growth Phase, Metastatic Melanoma

- **Layers**: Epidermis, Basement membrane, Dermis, Subcutaneous tissue and blood vessels

- **Metastasis**: to other tissues
BRAF

- BRAF gene is a proto oncogene located on the long arm of chromosome 7 at position q34.
- Mutations in BRAF are present in many types of cancer, including malignant melanoma
- The BRAF gene codes for a signalling protein
- It consists of 18 exons which give origin to a serine / threonine kinase belonging to the RAF protein family with A-RAF and C-RAF.

### BRAF gene (exon 15)

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Why sequence melanomas?

- The most frequent mutations determine an exchange between the Valine (V), in position 600 in exon 15 of the gene and the 'glutamic acid (E) (or lysine (K)).
- V600E mutation discovered in 2002 at the Sanger Institute and the Institute for Cancer Research
- Identifying a commonly occurring mutation in a cancer gene can provide a potential new drug target
BRAF mutations in human cancer

June, 2002 BRAF mutations in 7% of cancer
60% of melanoma
a Physiological conditions

Growth factor

RTK

PI3K
AKT
GRB2
SPRY
SOS
RAS
RAF
RAF
MEK
ERK

Transcription factors

CCND1
DUSP
SPRY

Cell cycle progression
Feedback

b BRAF V600E tumors

V600E
RAF
MEK
ERK

Transcription factors

CCND1
DUSP
SPRY

Increased proliferation
Strong feedback
**BRAF mutations**

- In which tissues are *BRAF* mutations most commonly found?
  - thyroid
  - large Intestine
  - skin
  - Ovary
  - Hairy cell leukemia
BRAF V600

PCR Real-Time

DNA Sequencing

Pyrosequencing

BRAF 1799 T>A V600E
BRAF and Dabrafenib

- **BRAF**
  - Protein kinase
  - Active in regulating RAS/RAF/MEK/ERK signaling pathway, which regulates cell growth
- **BRAF** mutations commonly cause cancer¹

- **Dabrafenib**
  - Orally available, selective ATP-competitive inhibitor of BRAFV600-mutated kinase
  - Approved in multiple countries in adults with unresectable or metastatic BRAFV600-mutated melanomas
    - Approval based on pivotal phase 3 clinical trial (BREAK-3)²
    - Adult dose of 150 mg capsule twice a day based on pharmacokinetics, pharmacodynamic endpoints³, and favorable benefit risk²

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Vemurafenib function

- Growth factor
- Growth factor receptor
- Ras
- Adapter proteins
- Vemurafenib
- BRAF^{V600E}
- MEK
- ERK
- Transcription factors
- Nucleus
- Growth, proliferation and survival

Zelboraf (vemurafenib)
Trametinib: A First in Class Oral MEK Inhibitor

- Trametinib is a highly selective reversible allosteric inhibitor of MEK1 and MEK2 activity.
- Trametinib inhibits RAF-dependent phosphorylation of MEK1.
**Resistance mechanisms**

1. Mutation in RAS leads to signalling through RAF/MEK/ERK
2. Overexpression of other RAF kinases, e.g. CRAF, leads to signalling through MEK/ERK
3. Mutations in MEK, downstream of RAF, leads to signalling through ERK
4. Activation of other signalling pathways, e.g. PI3K/Akt

**Key Components**
- Growth factor receptor
- Growth factor
- Adapter proteins
- RAF
- MEK
- ERK
- Transcription factors
- Akt
- Survival factors (e.g. IGF1)

**Drugs and Mutations**
- Vemurafenib (BRAFV600E)
- RASQ61K
- MEK121S

**Outcomes**
- Growth, proliferation and survival
Immunotherapy in Malignant Melanoma
Immune System Cells and Tumor Microenvironment in Melanoma

The health of the immune system critically regulates tumor growth within microenvironment by activating the innate and adaptive immunity.

Antigen Presenting Cells
- Tumor-associated Macrophages (TAM)
- Regulatory T cells (TREGs)
- Cytotoxic CD8^+ T-cells
- Natural Killer cells (NK)

Tumor Cells
- Tumor Antigens

Adaptive Immunity
- Dying cancer cell
- Antibody
- Engulfed dying cancer cell
- MHC molecule
- IL-12
- IL-18
- CD40L
- CD4^+ T cells
- CD8^+ T cells
- NK cell
- Migration
- Maturation

The immune escape of tumor cells and the defective adaptive response may be restored by immunotherapy.

Dranoff G, Nat Rev Cancer 2004
Attiva Windows Date & Impostation
What are Immune Checkpoints (ICP)?

The first and well described immune checkpoints: CTLA-4 and PD-1
Co-stimulation via CD28: T-cell activation

CTLA-4 blocks co-stimulation: No T-cell activation

Ipilimumab blocks CTLA-4: T-cell activation
**MoA PEMBROLIZUMAB**

**PD-1 checkpoints pathway**

- **PD-L1**
- **Tumor antigen presentation**
- **T cell activation in the lymph node**

**Dendritic cell** (professional antigen presenting cells, APC)

**Tumor derived antigens**

**Killer T cell reactivation**

- **CD8+ Killer T cells** (Cytotoxic T lymphocyte, CTL)
- **Perforin**
- **Granzyme**
- **IFN-γ**
- **IL-2**

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Tumor antigen presentation

Dendritic cell (professional antigen presenting cells, APC)

Tumor derived antigens

T cell activation in the lymph node

BLOCCO della immunosorveglianza

Killer T cell reactivation

CD8+ Killer T cells (Cytotoxic T lymphocyte, CTL)

Perforin
Granzyyme
IFN-γ

IL-2

Dendritic cell (professional antigen presenting cells, APC)

Tumor antigen presentation

Tumor derived antigens

Pembrolizumab

Killer T cell reactivation

CD8+ Killer T cells (Cytotoxic T lymphocyte, CTL)

Perforin
Granzyme
IFN-γ

Prevents PD-L1-mediated CTL anergy/exhaustion/apoptosis

T cell activation in the lymph node

Tumor

Pembrolizumab (MK3475) è un anticorpo monoclonale IgG4, umanizzato, ad alta affinità, anti PD-1

Similar reactivity to human and other primate PD-1, no reactivity to mouse or rat PD-1

Bloccando l’interazione tra PD-1 e i suoi ligandi naturali (PD-L1 or PD-L2) rompe il meccanismo di immunotolleranza nei confronti del tumore e riattiva l’immunosorveglianza