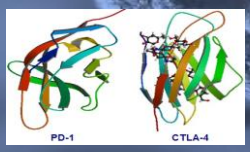

IMMUNOSUPPRESSION INDUCED BY CANCER CELLS: PD-1 AND CTLA-4 PATHWAYS

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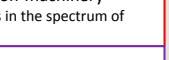
INTRODUCTION

○Cancer cells induce **immunologic tolerance** → absence of an immune response against certain antigens, causing the escape of these cells from the immune system.
○ 2 groups of tumor antigens: true tumor-specific antigens (encoded by mutant cellular genes), and tumor-associated antigens (encoded by normal cellular genes).

IMMUNOSUPPRESSIVE STRATEGIES EMPLOYED BY TUMOR CELLS TO EVADE T-CELL IMMUNITY

1. Downregulation of the Ag presentation machinery
   Absence/↓ MHC-I expression and changes in the spectrum of peptides presented by MHC-I
2. Defects in proximal TCR-mediated signaling
   ↓ expression of CD3ζ chain, p56lck and p59fyn in lymphocytes
3. Secretion of immunoregulatory cytokines
   TGFβ, IL-10, prostaglandin E2 and sialomucins
4. **Negative costimulatory pathways**
   CTLA-4 and PD-1
5. Modulation of tryptophan catabolism
   Indoleamine 2,3-dioxygenase overexpresse
   tumor cells → tryptophan depletion
   

6. "Tumor counterattack hypothesis"
   Death signals to T-cells through FasL/TRAIL-dependent mechanism
7. Regulatory T-cells
   Under the influence of CCL22 Tregs migrate into tumor site

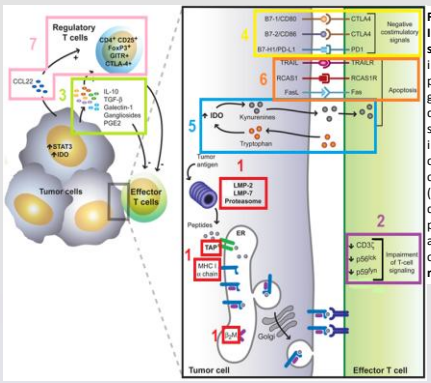


Figure 1. Immunosuppressive strategies. (1) impairment of the Ag presentation/processing machinery, (2) defects in TCR proximal signals, (3) secretion of immunoregulatory cytokines, (4) negative costimulatory signals, (5) tryptophan depletion, (6) proapoptotic pathways, and (7) regulatory T-cells. **Adapted from reference [2].**

PD-1 PATHWAY

❖ Programmed cell death-1 is a 288 aa protein that belongs to Ig superfamily. Consists in an extracellular IgV-like domain, and a cytoplasmic region (with an ITIM and an ITSM). — regulator of T-cell function (figure 2).
❖ Ligands: PD-L1 (B7-H1) and PD-L2 (B7-DC), expressed in lymphoid, in non-lymphoid cells and in tumor cells.

Figure 2. Human programmed cell death 1 receptor structure. Reference [8].

PD-1 signaling

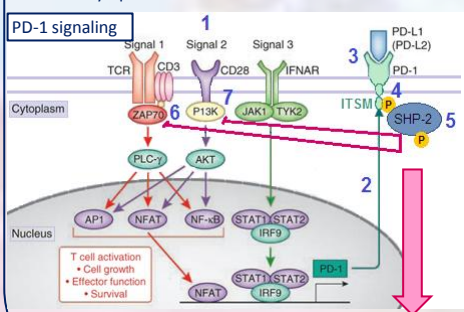


Figure 3. PD-1 signaling. (1) T-cell activation, (2) PD-1 expression, (3) PD-1/PD-L interaction, (4) ITSM phosphorylation, (5) SHP-2 recruitment and phosphorylation, (6+7) dephosphorylation of proximal and downstream effector molecules. **Adapted from reference [10].**

*It have been observed in PD-1 KO mice the loss of peripheral tolerance and the development of autoimmunity.

Apoptosis, anergy, exhaustion, molecular shield, IL-10 production → **T-cell tolerance**

CTLA-4 PATHWAY

❖ CTLA-4 (Cytotoxic T-lymphocyte associated antigen protein 4), is a 223 aa glycoprotein that belongs to Ig superfamily and has an extracellular IgV-like domain. — regulator of T-cell activation (figure 4).
❖ Ligands: B7-1 (CD80) and B7-2 (CD86), expressed in APCs and in tumor cells.

Figure 4. Solution structure of human CTLA-4 with a tetrasaccharide core attached. Reference [26].

CTLA-4 signaling

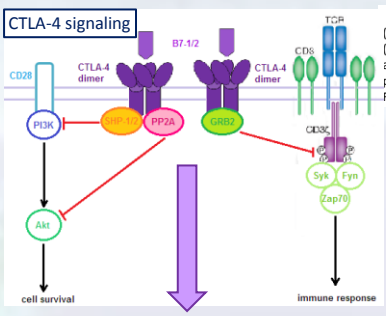


Figure 5. Activated CTLA-4 binds to: (1) SHP1/2 → PI3K dephosphorylation, (2) PP2A → ↓ Akt phosphorylation, and (3) TCR-CD3 complex → no phosphorylation → no Zap70, Syk and Fyn binding.

*CTLA-4 KO mice developed autoimmune diseases and died at 3-4 weeks old → significant role in the development of peripheral tolerance to self-proteins.

Prevention of T-cell activation through proximal and distal mechanisms

DIFFERENCES BETWEEN PD-1 AND CTLA-4

	CTLA-4	PD-1	Implications
Expression	T-cells	T-cells, B-cells and myeloid cells	PD-1: more broadly role in the regulation of immune responses
Interaction with AP2 (endocytosis)	Yes	No	CTLA-4 undetectable on the cell surface; PD-1 greater expression
SHP-2 association	Indirectly	Directly	CTLA-4 preserves a little PI3K activity, whereas PD-1 affects a more global inhibition of T-lymphocytes (figure 6)

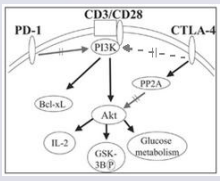


Figure 6. T-lymphocyte inhibition by CTLA-4 and PD-1. CTLA-4 preserves some PI3K activity but inhibits Akt directly. PD-1: inhibits PI3K directly. **Adapted from reference [35].**

IMPLICATIONS FOR CANCER IMMUNOTHERAPY

A variety of checkpoint blocking agents have been developed to block PD-1 and CTLA-4 signaling (including monoclonal Ab). Examples: Nivolumab and Lambrolizumab (anti-PD-1), Ipilimumab (anti-CTLA-4).

PROS
✓ More effective than classical therapies in metastatic cancers.
✓ Synergistic activity → combinatorial therapies.

CONS
✘ Side effects.
✘ Very new, we don't know the long-term side effects.
✘ Currently very expensive therapies → not accessible to everyone.

CONCLUDING REMARKS

1. **PD-1 and CTLA-4:** powerful negative regulators of the immune response (KO experiments, development of autoimmunity) → very **important role in the regulation of the IS**, immunosuppression.
2. **Cancer** uses this mechanism to **evade** and **escape from IS cells → tolerance** toward cancer cells, leading to the development of malignant tumors.
3. **Therapy:** it's difficult to find a monotherapy that works at 100% because there are many different molecules and mechanisms involved, → **combinatorial therapies** should be tested.

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