Laboratory of Tumour Immunology

Anti-tumour immunotherapy, immunoediting, immunoepigenetics

Milar Reiniš

milan.reinis@img.cas.cz

www.img.cas.cz/research-groups/milan-reinis

The capacity of tumour cell populations to escape from antitumour immunity in the course of tumour development represents a serious obstacle to the development of effective anti-tumour immunotherapy or vaccination. In our laboratory, we are mainly focused on selected reversible processes, such MHC class I deficiency or altered expression of co-stimulatory/inhibitory molecules, by which tumour cells can escape from specific immune responses. In last several years, we have been interested in epigenetic mechanisms underlying reversible MHC class I downregulation on tumour cells, as well as in the design of immunotherapy/vaccination that would be effective against MHC class I-deficient tumours. Using murine models for MHC class I-deficient tumours (e.g. cervical carcinoma, prostate cancer), in which the MHC class I expression could be restored by cytokines, we have documented association of the MHC class I cell surface expression and DNA methylation of the regulatory regions of the antigen-presenting machinery genes. We have also found that DNA methyltransferase inhibitors induced expression of the genes involved in antigen-processing machinery and surface expression of MHC class I molecules on tumour cells, as well as of selected co-stimulatory and inhibitory molecules. In vivo experiments documented the efficacy of immunotherapy of MHC class I-deficient tumours combined with administration of 5-azacytidine, a DNA methyltransferase inhibitor. Our results also suggest an important role of the DNA methylation in the interferon γ-induced expression of antigen-presenting machinery genes. We are also interested in epigenetic mechanisms underlying regulation of genes encoding antigen-presentation machinery genes, as well as co-stimulatory/inhibitory genes in antigen-presenting or regulatory immunocytes. Besides DNA methyltransferase inhibitors, we have investigated other immunomodulatory chemotherapeutics, such as cyclophosphamide, especially in terms of the impacts of immunoactive molecule expression on tumour cells. Further, our areas of interest are populations of immunoregulatory cells and their dynamics and function in the course of chemotherapy. Recently, we have characterized in detail the immunosuppressive character of myeloid-derived suppressor cells induced by cyclophosphamide administration in mice.
In our projects we have been interested in experimental anti-tumour immunotherapy and vaccines, with a special attention paid to the minimal residual tumour disease treatment. We have used cell and gene therapy approaches and dendritic cell-based vaccines, as well as genetically modified tumour cells producing cytokines (especially IL-12-producing cells) for vaccination and immunotherapy optimization.

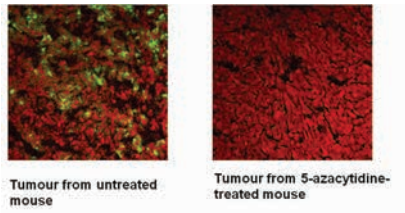

Fig. 1. Fluorescence staining of Gr-1-positive tumour infiltrating cells in tumours. Sections from large (cca 1.5 cm diameter) tumours derived from untreated and 5-azacytidine-treated (i.p. one 200 mg dose) were stained for the presence of Gr-1 marker-positive cells. Most of these cells represent myeloid-derived suppressor cells. Their number in the tumour microenvironment upon the treatment of mice with 5-azacytidine decreased.

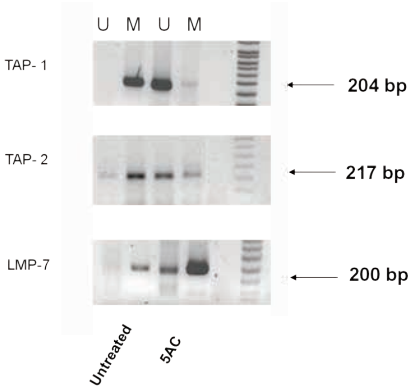

Fig. 2. Methylation specific PCR (MSP) analysis of selected antigen presenting genes promoter sequences in tumour cells from mice treated with 5-azacytidine. DNA from the TC-1/A9 explanted tumour cells from untreated and 5AC-treated animals was bisulphite treated and subjected to the MSP analysis. The methylation status of the representative antigen-presenting machinery genes *TAP-1*, *TAP-2* and *LMP-7* promoter sequences was analyzed. Bands in the lanes designated U represent the PCR products amplified from unmethylated DNA, bands from the M lanes represent the PCR products from methylated DNA.

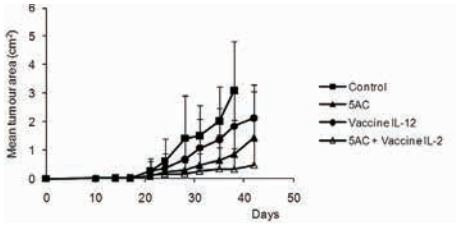

Fig. 3. Combined therapy of murine MHC class I-deficient TC-1/A9 tumours with IL-12-producing vaccine (irradiated TC-1/IL-12 cell line) and 5-azacytidine. Tumour growth curves in control mice and mice subjected to chemo- and immunotherapy and their combination are presented. TC-1/A9 tumour cells were transplanted into syngeneic mice on day 0 and 5-azacytidine (5AC) was repeatedly administered on days 3, 7, 10, 14, 17, 21, 24 and 28, IL-12-producing cells (Vaccine IL-12) were administered on day 4. Significant inhibition of the tumour growth was observed in all treated mice, as compared to the untreated controls. Moreover, combined chemoimmunotherapy was more effective, as compared to monotherapies only.

- EU FP, Network of Excellence, 018933 - Clinigene, European Clinical Gene Transfer Advisory Network, 2006-2011, J. Bubeník
- GA CR, GA301/07/1410 - Immunosuppressive cell populations in the course of tumour progression and therapy, 2007-2011, M. Reiniš
- Ministry of Health of the Czech Republic, NS10660/2009 - Development of experimental anti-WT 1 vaccine for immunotherapy of tumours, 2009-2011, M. Indrová (co-investigator)
- GA CR, GA301/09/1024 - Molecular and cellular mechanisms of tumour chemotherapy: immunomodulatory effect, 2009-2011, M. Indrová
- GA CR, GAP301/10/2174 - Epigenetic mechanisms in regulation of genes important for antigen presentation and antitumour immunity, 2010-2013, M. Reiniš
- GA CR, GPP301/11/P220 - Mechanisms underlying cyclophosphamide-induced accumulation of myeloid derived suppressor cells, 2011-2013, R. Mikyšková

1. Mikyšková R, Indrová M, Polláková V, Bieblová J, Šímová J, Reiniš M. Cyclophosphamide-induced myeloid-derived suppressor cell population is immunosuppressive but not identical to myeloid-derived suppressor cells induced by growing TC-1 tumors. **J Immunother 2012** 35(5): 374-384.
2. Štěpánek I, Indrová M, Bieblová J, Fučíková J, Spíšek R, Bubeník J, Reiniš M. Effects of 5-azacytidine and trichostatin A on dendritic cell maturation. **J Biol Regul Homeost Agents 2011** 25(4): 517-529.
3. Šímová J, Polláková V, Indrová M, Mikyšková R, Bieblová J, Štěpánek I, Bubeník J, Reiniš M. Immunotherapy augments the effect of 5-azacytidine on HPV16-associated tumours with different MHC class I-expression status. **Br J Cancer 2011** 105(10): 1533-1541.
4. Indrová M, Šímová J, Bieblová J, Bubeník J, Reiniš M. NK1.1+ cells are important for the development of protective immunity against MHC I-deficient, HPV16-associated tumours. **Oncol Rep 2011** 25(1): 281-288.
5. Mikyšková R, Indrová M, Šímová J, Bieblová J, Bubeník J, Reiniš M. Genetically modified tumour vaccines producing IL-12 augment chemotherapy of HPV16-associated tumours with gemcitabine. **Oncol Rep 2011** 25(6): 1683-1689.

From the left:
Ivan Štěpánek, MSc / PhD Student · Veronika Mayerová, MSc / PhD Student (from 2012) · Marie Indrová, PhD / Research Fellow · Jana Šímová, PhD / Research Fellow · Veronika Polláková, MSc / PhD Student ·Romana Mikyšková, MD, PhD / Research Fellow · Milan Reiniš, PhD / Head of Laboratory · Zuzana Paračková / Diploma Student · Martin Šrámek, MSc / PhD Student (from 2012) · Jana Bieblová, MSc / Research Assistant · Magdalena Cebová / Diploma Student · Renata Turečková / Technician

Not in the picture:
Veronika Hrušková / Diploma Student · Anna Žlabová / Diploma Student (until 2012) · Prof Jan Bubeník, MD, DSc (until 2011)