**Institutionen för Onkologi-Patologi**

# Interactions between neuroblastoma and the immune system –

## cellular pathways and mediators

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Radiumhemmets föreläsningssal, Karolinska Universitetssjukhuset, Solna

**Fredagen den 25 mars 2011, kl 09.30**

av
**Lena-Maria Carlson**
MD

*Huvudhandledare:*
Professor Per Kogner
Karolinska Institutet
Institutionen för Kvinnor och Barns Hälsa

*Bihandledare:*
Professor Rolf Kiessling
Karolinska Institutet
Institutionen för Onkologi-Patologi

*Bihandledare:*
Dr Philos Baldur Sveinbjörnsson
Karolinska Institutet
Institutionen för Kvinnor och Barns Hälsa

*Fakultetsopponent:*
Professor Holger Lode
Department of Pediatrics
University of Greifswald, Germany

*Betygsnämnd:*
Docent Ola Winqvist
Karolinska Institutet
Institutionen för Medicin

Professor Magnus Essand
Uppsala Universitet
Institutionen för Immunologi, Genetik och Patologi

Docent Anna Nilsson
Karolinska Institutet
Institutionen för Kvinnor och Barns Hälsa

**Stockholm 2011**

# ABSTRACT

Neuroblastoma (NB) is an embryonal tumor of early childhood arising in tissues of the sympathetic nervous system, such as the adrenal gland and paraspinal ganglia. It is the most common extra-cranial solid tumor of childhood, and 10-20 children are diagnosed with NB each year in Sweden. The overall survival rate is about 70%, but 50% of the children in the high-risk group succumb in spite of intense multimodal therapy. This warrants the search for alternative treatment strategies. One upcoming treatment option is immunotherapy, which represents a specific treatment modality with the possibility of minimizing long-term side effects in survivors.

Cellular therapies for NB have previously been discouraged due to the notion that NB is a tumor of low immunogenicity. This thesis demonstrates that differentiating agents alter the immune phenotype of primary NB tumors and cell lines such as to enhance the expression of classical HLA molecules and the adhesion molecule ICAM-1. This was paralleled by an increased ability of differentiated NB cells to bind granzyme B at the cell surface and translated into enhanced killing by natural killer (NK) cells and T-cells. These results argue in favor of differentiation and cellular immunotherapy as a combined auxiliary approach for NB patients (paper I). Furthermore, the work presented in this thesis demonstrates that tumor-non-specific activated cytotoxic T lymphocytes (CTLs) release effector molecules which facilitate immune-mediated recognition of NB. Effector molecules from CTLs upregulated HLA class I, ICAM-1 and Fas at the cell surface and restored the expression and activity of caspase-8 in primary NB tumors and cell lines. This rendered NB cells more susceptible to death receptor-mediated killing (paper II).

This thesis also demonstrates that primary human NB samples, representing all genetical subtypes, harbor tumor-infiltrating T-cells which proliferate *in situ*. Tumor-infiltrating lymphocytes were preferentially $CD8^+$, expressed high levels of the activation marker CD25 and exhibited a phenotype of memory cells. Autologous peripheral blood lymphocytes were exposed to tumor cells *in vitro* and their production of IFN-γ and TNF-α was increased, while an activated phenotype was obtained. This indicates that human NB cells do not prevent the generation of active T-cell responses (paper III). In the transgenic TH-MYCN mouse model of NB, tumor-associated inflammation was investigated and NB tumor progression was shown to be paralleled by a gradual suppression of intratumoral T-cell responses in favor of immature cells of the innate immune system. Anti-inflammatory treatment with low-dose aspirin displayed a promising efficacy in delaying tumor outgrowth with a concomitant abrogation of an inflammatory switch (paper IV).

Taken together, the work presented in this thesis demonstrates that NB can serve as a proper target for cellular immunotherapy. It argues for an early implementation of immunotherapy in clinical protocols, where differentiating agents and/or the attraction of activated CTLs to the NB microenvironment could enhance immune-mediated tumor recognition.



ISBN 978-91-7457-229-2