ABSTRACT – David Větvička, M.Sc

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Many researchers have, in the past, focused on pathophysiological features of tumor tissue, various tumor-nonmalignant cell interactions, and secretion of active molecules within the tumor mass. All these aspects of tumor structure are known as tumor microenvironment. The composition of particular tumor ecosystem is highly variable, with differences between various tumor types, even between patients with the same diagnosis, and in separate areas of the same tumor. Moreover, further changes in tumor microenvironment often occur during the progression of the disease. Studies of tumor microenvironment have revealed both novel targets for therapy and new prognostic markers. New therapy modalities are being developed to target these discovered features, including drugs functioning to boost anti-malignancy immunity, to block pro-metastatic potential, or to utilize the unique features of this pathological environment established by the tumor. These are obviously of great interest and harbor high potential for better management of malignant diseases.

The focus of this thesis is to study the interactions of polymeric drug delivery systems within the tumor microenvironment and to utilize various features of this specific niche for drug delivery research. We have followed three direction related by polymeric drug delivery systems: A) the linear HPMA copolymeric carriers, their impact on the tumor microenvironment and targeting to specific features of the tumor microenvironment, B) degradable polymeric micellar carriers for treatment and diagnosis of cancer – utilization of the EPR Effect and C) establishment of an imageable T-lymphoma cell line, as a tool for better insight into drug delivery research.

We studied the changes in glycosylation paterns of cancer cells after treatment with free doxorubicin and two polymeric conjugates. Polymeric conjugates differed in the way of conjugation of doxorubicin, as it is bound *via* an amide bond (Dox-HPMA^{AM}), or *via* hydrazone pH sensitive bond (Dox-HPMA^{HYD}). Neither the free drug nor Dox-HPMA^{HYD} have any influence on cellular surface glycosylation. But the Dox-HPMA^{AM} conjugate enhanced expression of CD43 molecule and also altered the whole glycosylation pattern of EL-4 cells.

The HPMA conjugate targeted by cyclo(RGDfK) peptide, has shown excellent binding capacity to the cells expressing $\alpha_V\beta_3$ integrin. We have also proved significant *in vivo* accumulation of by cyclo(RGDfK) targeted conjugate (contrary to conjugate with scrambled oligopeptide) at the tumor periphery (EL4; nonexpressing $\alpha_V\beta_3$ integrin) in the area of intensive neoangiogenesis. Such conjugate can abolish neoangiogenesis by the blocking of integrin binding with targeting peptide and simultaneously release the cytostatic drug payload from its polymeric backbone and kill tumor cells directly.

Tested micellar delivery system for doxorubicin, which is bound into the micellar core through a hydrolytically cleavable hydrazone bond. System performed a long circulation time and has a very low systemic toxicity. We have recorded promising therapeutical efficiency against experimental murine cancer. We also documented activation of the immune system during the treatment, and we can state that usage of micelles triggers the establishment of an effective tumor-specific immunological memory.

Moreover, we prepared a two thermoresponsive, hydrolytically degradable, micellar delivery systems for possible use in radiodiagnostics and radiotherapy. Studied polymers form micelles after rapid heating to 37ºC. The usage of a hydrolytically degradable *N*-glycosylamine bond allowed slow degradation, which leads to the production of polymeric blocks sized under the renal threshold. The polymers showed no direct cytotoxicity against tested murine and human cell lines, so it could be considered as a promising delivery system.

Two different stable EGFP transfectants (clones 3 and 12) of murine T-cell lymphoma (EL-4) were established and characterized. Clone 3 exhibits similar tumor growth rate in immunocompetent mice as parental cell line, this makes it a usefull tool for evaluating the interaction of the immune system in response to the treatment. Clone 12 possesses a very bright signal, which enables the tracking of metastatic spreading in immunodeficient murine models.