Molecular Dissemination into Local and Systemic Tissues is Regulated by Molecular Size and Tumor Vascular Status: Implications for Targeted Drug Delivery in Cancer Immunotherapy

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Statement of Purpose: Tumors secrete biomolecules (proteins, exosomes) of a broad range of physiochemical properties (chain-like biopolymers, membrane-enclosed particulates) and sizes (from 1-100 nanometer (nm) and greater in diameter) that are involved in tumor immune suppression. Since normal blood capillaries are permeable only to low molecular weight (MW) solutes (<5 nm in diameter), lymphatic drainage supports the clearance of intermediate MW solutes (>40kDa or >5nm diameter but <100 nm) from the intercapillary tissue space, and cell-mediated trafficking clears large particulates (>100 nm), molecular size characteristics are hypothesized to crucially influence molecular clearance rate, partitioning between blood and lymphatic routes, and the capacity of tissue-produced molecular species to disseminate systemically. However, though the tumor vascular plexus is well described to be irregular and highly permeable, how it influences the extent and rate of molecular clearance as well as blood versus lymphatic apportionment of low versus high MW tumor-derived molecules remains undetermined. This has important implications for the distribution of immune modulatory antigens and cytokines from the tumor microenvironment to disseminated tissues and in the development of targeting strategies to mitigate their signaling effects. We therefore sought to evaluate the contribution of the developing tumor vasculature on molecular clearance and dissemination patterns by comparing the clearance and biodistribution of a panel of near-infrared fluorescently-labeled molecules from healthy versus malignant tissues.

Methods: The near-infrared molecular beacon panel was comprised of dextrans and polystyrene nanospheres chosen as non-degradable, physicochemical representatives of cell-derived molecules of interest from 5-500 nm in diameter. Moreover, these molecules were labeled with near-infrared NHS-ester dyes in order to minimize spectral overlap among beacons and increase the limit of detection in tissue homogenate. C57Bl/6 mice were intradermally injected with the beacon panel and tissues (lymph nodes, lungs, spleen, liver, kidneys, tumor, etc.) were harvested and individually homogenized at 1, 4, 24, and 72 hours post-injection to observe the transport of the beacons from healthy dermal tissue. Alternatively, mice were implanted with B16F10 melanoma on day 0 and the beacon panel was injected intratumorally in groups 5, 7, or 9 days later to observe the effect of abnormal tumor vasculature on the clearance and biodistribution of tumor-derived molecules by means of fluorescence detection in tissue homogenate.

Results: The rapid growth B16F10 melanomas in C57Bl6 animals resulted in significantly increased vascular surface area and a disorganized vascular plexus within seven days of tumor implantation (Fig. 1A). The rate of clearance of 30 nm dextran from the tumor interstitium was slowed by 10 fold from day 7 tumors and 20 fold from day 9 tumors as compared to naïve dermal tissue; additionally, lymphatic-mediated transport to sentinel lymph nodes was attenuated by malignancy (Fig. 1B). Interestingly, tumor growth increased blood permeability at early-stages, with the day 5 and 7 tumor vasculature enabling 500 nm spheres and 30 nm dextran to disseminate systemically within one hour of injection. Consequently, the liver demonstrated increased exposure to high-MW (500-30 nm) molecules escaping from early-stage tumors (Fig. 1C), which was absent in well-developed, day 9 tumors as well as normal, healthy tissues. Small, 5 nm dextran exhibited increased systemic bioavailability from day 7 and 9 tumors, with a significantly larger accumulation in the liver versus kidney for the day 7 v 9 tumors, respectively.

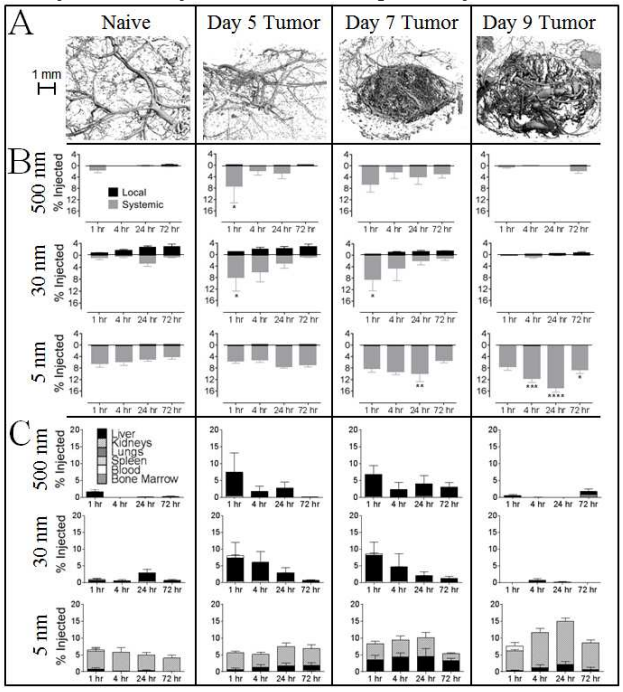

Figure 1: A) Micro-computed tomography of contrast-agent filled vessels within B16F10 melanomas. B) Tumor growth reduces local (sentinel lymph node) beacon accumulation and increases their systemic (spleen, lung, liver, kidney, blood, and bone marrow) distribution. C) Molecular size regulates beacon accumulation in disseminated systemic tissues.

Conclusion: Vascular remodeling associated with malignant disease progression redirects molecular dissemination patterns from tumors. These results have important implications for the disrupted intracellular signaling involved in directing immune suppression versus immunity in cancer as well as the targeted delivery of immunotherapeutic drugs for management of disease.