MSLN-TARGETED IMMUNOTOXIN LMB-100 INDUCES DEVELOPMENT OF TERTIARY LYMPHOID STRUCTURES AND TUMOR REGRESSIONS OF ORTHOTOPIC MESOTHELIOMA

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Background Recombinant immunotoxins (RITs) are chimeric proteins composed of an Fv linked to a toxin and kill cells by arresting protein synthesis. LMB-100 is an RIT that kills mesothelin (hMLSN) expressing cells. Mesothelin is expressed in mesothelioma and many other cancers. Clinically, we observed some patients had delayed responses to LMB-100, suggesting the induction of antitumor immunity. The present study has developed a transgenic mouse model to investigate if LMB-100 monotherapy eradicates orthotopic mesothelioma and induces anti-tumor immunity in mice, and to study the mechanism of this immunity.

Methods To study the immune response induced by LMB-100, we established an immunocompetent transgenic mice model that expresses hMLSN only in the thyroid gland, so that hMLSN-expressed cells won’t be rejected. AB1-L9 mouse mesothelioma cells expressing hMLSN were injected into the peritoneal cavity. Mice were treated with LMB-100 (i.p.) and mice with complete responses (CRs) were rechallenged with tumor cells (s.c.) to determine if anti-tumor immunity has developed. Tumors were analyzed by Nanostring and protein arrays, and the distribution of various immune cells was assessed by immunohistochemistry.

Results LMB-100 treatment alone induced CRs in 19/35 mice. Importantly, the CR mice were protected from re-challenge with AB1-L9 cells (17/19 mice), indicating LMB-100 induces anti-tumor immunity in mice. Both non-targeted immunotoxin (LMB-34) and inactive immunotoxin (LMB-255) had no anti-tumor activity, indicating both cell targeting and killing are necessary. The CRs were blocked by antibodies that deplete CD8+ T cells, CD4+ T cells as well as B cells. We further analyzed regressing tumors and found that pathogen response pathways are activated as early as 12 hours, followed by upregulation of a cascade of immune-related pathways associated with chemotactic-related activities (cytokine & receptors), antigen presentation (DC function), and TIL-based cell killing activities (T cell functions). We observed tertiary lymphoid structures (TLS) with aggregations of B cells developed in the tumor. Genes encoding proteins that are responsible for chemotaxis signals, such as CCL20, CXCL13, and CCL21, were specifically increased in tumors stroma, but not in tumor cells treated in vitro.

Conclusions We show RIT monotherapy gives complete regressions of tumors and reprograms the tumor immune microenvironment to trigger anti-tumor immunity. The regression of the primary tumor requires CD8+ T cells, CD4+ T cells as well as B cells. LMB-100 mediated tumor cell death induces the development of TLS in the solid tumor, probably by upregulating chemotaxis chemokines and cytokines that are associated with TLS formation.