Phosphoantigenic activation of Vγ9Vδ2 T lymphocytes: involvement of RHOB in a lung cancer model

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**Purpose:** Lung cancer is the leading cause of cancer-related mortality worldwide. Recently, immune checkpoint blockade have demonstrated promising antitumor effects in non-small cell lung cancer (NSCLC) patients and are currently being investigated. However, it remains innate or adaptive resistance mechanisms. Active immunotherapy such as adoptive T cell-transfer represents one promising approach and Vγ9Vδ2 T lymphocytes are attractive candidates. These cells are able to be activated by synthetic soluble phosphoantigens (PAg) and by endogenous PAg such as some intermediates of the mevalonate/cholesterol pathway in mammalian cells and which can be overexpressed in modified cells [1]. However, the molecular determinants required for activation of Vγ9Vδ2 TCR on target cells have long remained elusive. Recent works identified the membrane-expressed butyrophilin BTN3A1 as a key molecule in PAg-induced activation of Vγ9Vδ2 T cells [2] and the RAS-related small GTPase RHOB mediating endogenous PAg recognition by the Vγ9Vδ2 TCR [3] but the mechanism involving these two molecules is still unclear.

RHOB is involved in cytoskeleton rearrangement, intracellular trafficking, migration and invasion, and displays tumor suppressor activity in NSCLC as its expression is often downregulated in aggressive tumors [4]. How RHOB exerts this tumor suppressor behavior is still poorly understood. Thus, we formulated the hypothesis that loss of RHOB expression in lung cancer cells could impair the response of the γδ T cells.

**Experimental Design and results:** For this project, we analyzed the reactivity of primary Vγ9Vδ2 T cell lines in coculture with a panel of NSCLC cell lines proficient or not for RHOB (RHOB KO), that harbor different oncogenic driver mutations commonly found in lung cancers (i.e. EGFR and KRAS mutations). We measured Vγ9Vδ2 T degranulation, cytokines expression and trogocytosis by cytometry and microscopy analysis using different blocking strategies of the PAg activation. Our first data indicate that loss of RHOB expression in NSCLC cells decreased γδ T activation according to the oncogenic context of the target, suggesting a differential role of RHOB according to the mutational status.

**Conclusion:** This study could open new strategies of treatments for NSCLC using γδ T cell based immunotherapy.

**References:**