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Research Paper

Harnessing RNA sequencing for global, unbiased evaluation of two new adjuvants for dendritic-cell immunotherapy

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ABSTRACT

Effective stimulation of immune cells is crucial for the success of cancer immunotherapies. Current approaches to evaluate the efficiency of stimuli are mainly defined by known flow cytometry-based cell activation or cell maturation markers. This method however does not give a complete overview of the achieved activation state and may leave important side effects unnoticed. Here, we used an unbiased RNA sequencing (RNA-seq)-based approach to compare the capacity of four clinical-grade dendritic cell (DC) activation stimuli used to prepare DC-vaccines composed of various types of DC subsets; the already clinically applied GM-CSF and Frühsommer meningoencephalitis (FSME) prophylactic vaccine and the novel clinical grade adjuvants protamine-RNA complexes (pRNA) and CpG-P. We found that GM-CSF and pRNA had similar effects on their target cells, whereas pRNA and CpG-P induced stronger type I interferon (IFN) expression than FSME. In general, the pathways most affected by all stimuli were related to immune activity and cell migration. GM-CSF stimulation, however, also induced a significant increase of genes related to nonsense-mediated decay, indicating a possible deleterious effect of this stimulus. Taken together, the two novel stimuli appear to be promising alternatives. Our study demonstrates how RNA-seq based investigation of changes in a large number of genes and gene groups can be exploited for fast and unbiased, global evaluation of clinical-grade stimuli, as opposed to the general limited evaluation of a pre-specified set of genes, by which one might miss important biological effects that are detrimental for vaccine efficacy.

INTRODUCTION

Antigen presenting cells, such as Dendritic cells (DCs), play a central role in many immunotherapies because of their ability to induce immune responses or to promote immune tolerance by interacting with $CD4^+$ and $CD8^+$ T cells. For T cell activation to occur, DCs need to mature and migrate to the lymph nodes. DC immunotherapies aim to strengthen antitumoral immune responses by boosting T cell activation [1, 2]. In such therapies, DCs are isolated, activated and loaded with tumor antigen and then given back to the patient. Vaccine DCs are anticipated to promote antitumor responses by presenting tumor antigen in the context of costimulatory molecules and immune-stimulatory cytokines [3-7]. Upon activation, DCs upregulate costimulatory markers