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The ambiguous role of MyD88 in the regulation of lung cancer progression

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Continuous inflammatory conditions in the TME can induce cell stress, damage and death, resulting in the release of damage-associated molecular patterns (DAMPs). These molecular patterns are recognized by toll-like receptors (TLRs) and interleukin-1 receptor (IL-1R) families on innate cells, initiating a rapid immune response and the production of pro-inflammatory cytokines, chemokines and type I interferon. MyD88 is the canonical adaptor protein of TLR and IL-1R receptors and mediates the signaling from them. Its involvement in immune cells has been widely characterized, but MyD88 signaling also occurs in tumor cells. Unfortunately, the role played by MyD88 in the complex interaction between tumor cells, the immune system and the TME seems to be ambivalent. Some authors have demonstrated that MyD88 contributes to carcinogenesis, metastasis and immunosuppression. Others support its protective role in cancer models by promoting immune responses, as it empowers antigen presentation, maturation of dendritic cells and infiltration of T and NK cells. The causes and mechanisms by which the MyD88 balance moves to an anti- or pro-tumor outcome has not been deciphered so far and it is becoming evident that it has a dual functionality depending on the cell type, the binding receptor partners and the surrounding context. A comprehensive analysis of the role played by MyD88 in the complex interplay between cancer cells and the tumor stroma may unravel new prognostic, diagnostic and immunotherapeutic targets in lung cancer, the most deadly cancer type currently.

We analyzed in public tools based on The Cancer Genome Atlas (TCGA) the expression profile of MyD88 and found that MyD88 is mainly expressed by macrophages, dendritic cells and neutrophils, although tumor cells also express it. In LLC and KP murine lung cancer cell lines, we silenced MyD88 expression by CRISPR/Cas9 technology. We confirmed MyD88 knock out (KO) by western blot. By 3D cell culture, we noticed that MyD88 KO cells took more time than negative control cells (cells transfected with a non-target plasmid) to generate spheroids. Moreover, once formed, the spheroids were smaller or more disorganized in the MyD88 KO setting. Moreover, KP MyD88 wild-type (WT) and KO clones were characterized by bulk RNA-Seq, which revealed a total of 42 differentially expressed genes (29 down-regulated and 13 up-regulated) involved in cell metabolism, cell attachment, angiogenesis, growth factors and immune response. In order to study the role of MyD88 in tumor growth *in vivo*, LLC MyD88 WT and KO cells were subcutaneously injected in B6(C)/Rj-Tyr$^{c/c}$ mice. Unexpectedly, MyD88 deficiency enhanced tumor growth in this model as compared to control cells. The same results were obtained when KP cells were injected through the tail vein in an orthotopic model of lung cancer in B6(C)/Rj-Tyr$^{c/c}$ mice. These surprising results could be explained by the mutational status of these two cell lines (*KRAS* mutated, *TP53* silenced): anti-tumor effects of MyD88 silencing on *KRAS*-mutated tumor cells might be counterbalanced by the *TP53* deficiency.

In conclusion, these results provide evidence of the complex effects that MyD88 may have in lung cancer, which may be cell- and context-dependent. Shedding light to the role of MyD88 in lung tumor progression is required, since being a bottleneck in many inflammatory signaling cascades, its blockade may be a novel immunotherapeutic strategy.