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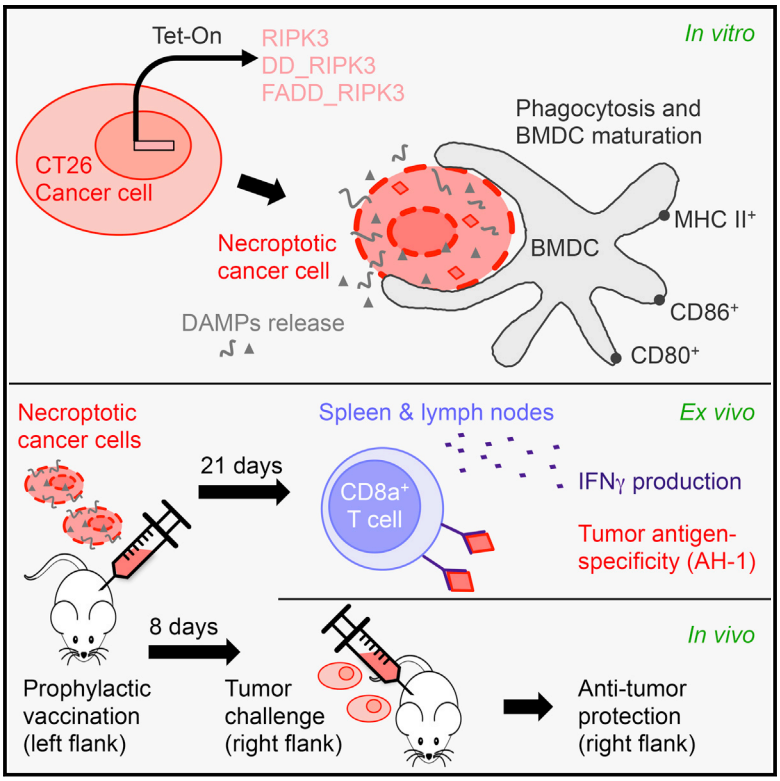
# Vaccination with Necroptotic Cancer Cells Induces Efficient Anti-tumor Immunity

## Graphical Abstract




## Highlights

- Necroptotic cancer cells release DAMPs and induce dendritic cell maturation in vitro
- Cross-priming of T cells was induced by necroptotic cancer cells in vivo
- Necroptotic cancer cells promote the tumor antigen-specific production of IFN-γ ex vivo
- Prophylactic injection of necroptotic cancer cells leads to an anti-tumor vaccination

## Authors

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## In Brief

Løve Aaes et al. show that necroptotic cancer cells induce the maturation of dendritic cells, the cross-priming of cytotoxic T cells, and the production of IFN-γ in response to tumor antigen stimulation. Using RIPK3 induction systems, the authors further demonstrate efficient prophylactic vaccination with immunogenic necroptotic cells.

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# Vaccination with Necroptotic Cancer Cells Induces Efficient Anti-tumor Immunity

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## SUMMARY

**Successful immunogenic apoptosis in experimental cancer therapy depends on the induction of strong host anti-tumor responses. Given that tumors are often resistant to apoptosis, it is important to identify alternative molecular mechanisms that elicit immunogenic cell death. We have developed a genetic model in which direct dimerization of FADD combined with inducible expression of RIPK3 promotes necroptosis. We report that necroptotic cancer cells release damage-associated molecular patterns and promote maturation of dendritic cells, the cross-priming of cytotoxic T cells, and the production of IFN-γ in response to tumor antigen stimulation. Using both FADD-dependent and FADD-independent RIPK3 induction systems, we demonstrate the efficient vaccination potential of immunogenic necroptotic cells. Our study broadens the current concept of immunogenic cell death and opens doors for the development of new strategies in cancer therapy.**

## INTRODUCTION

The discovery of immunogenic apoptosis (IA) underlines the importance of tumor-host interaction, in which the activation of an immune response, specifically toward malignant cancer cells, results in a potent and long-lasting anti-cancer immunity (Casares et al., 2005). IA is characterized by the release of damage-associated molecular patterns (DAMPs), such as cell-surface exposure of calreticulin (Garg et al., 2012a; Obeid et al., 2007a), secretion of ATP (Garg et al., 2012b; Ghiringhelli et al., 2009; Michaud et al., 2011), and release of the chromatin-binding protein high-mobility group B1 (HMGB1) (Apetoh et al., 2007; Yamazaki et al., 2014), each of which interact with the phagocytic or scavenger receptors, e.g., LRP1 (calreticulin), purinergic receptors (ATP), and pattern-recognition receptors, such as TLR4 (HMGB1), respectively. Thus, inducing IA in cancerous cells can be very beneficial in a therapeutic setting because DAMPs induce a host anti-tumor immune response. However, in order to overcome apoptosis resistance, which is often observed in tumors (Hanahan and Weinberg, 2011), it is of great importance to find other ways to kill tumor cells by triggering cell death modalities different from apoptosis.

Necrosis has long been described as a consequence of extreme physiochemical stress, such as osmotic shock and freezing-thawing (referred to throughout the text as "F/T"), and was therefore classified as uncontrolled or accidental cell death (Kaczmarek et al., 2013). However, more recent findings have demonstrated many different cellular stimuli that induce regulated forms of necrosis, which follow defined steps and signaling events reminiscent of a true cell death program. Necroptosis is one form of regulated necrosis and is mediated by receptor-interacting protein kinase-1 (RIPK1), RIPK3, and its substrate mixed lineage kinase domain-like (MLKL) and has been reported to contribute to inflammation under pathological conditions (Degterev et al., 2005; He et al., 2013; Holler et al., 2000; Pasparakis and Vandenabeele, 2015; Pierdomenico et al., 2014).

In this study, we evaluated the immunogenicity of necroptosis and tested its potential as an alternative approach in cancer therapy. We observed that cancerous CT26 cells undergoing necroptosis can be immunogenic in vitro and in vivo, where the necroptotic cells served as potent immunizers in a prophylactic tumor vaccination model. Vaccination with necroptotic cancer cells effectively cross-primed cytotoxic $CD8a^+$ T cells in vivo and elicited the strong CT26 tumor antigen-specific production of IFN-γ ex vivo. The immunogenicity of necroptotic cells did not correlate with the extent of NF-κB activation. These findings identify necroptotic cancer cells as efficient inducers of an adaptive immune response and show that necroptosis can be