SHORT COMMUNICATION

O-11. TLR3 MATURATION, LOCALISATION AND APOPTOTIC ROLE IN CANCER

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dsRNA, innate immunity, oral cancer, lung cancer.

Introduction
Double stranded RNA (dsRNA) is a nucleic acid intermediate produced during viral infection that can be sensed by innate immune receptors like TLR3. It has been clinically tested as a potential cancer treatment due to its immune stimulatory and growth inhibitory properties, and its low toxicity. Retrospective analyses of clinical trials have recently suggested that TLR3 expression by cancer cells could be a biomarker for its therapeutic efficacy (Salaun et al. 2011). We and others have shown that in addition to its role in immune responses, TLR3 can trigger caspase-8-dependent apoptotic cell death in various human cancer cells, providing a possible explanation for dsRNA efficacy in patients (Salaun et al. 2006). However, little is known about TLR3 protein trafficking, localisation, and post-translational modifications, which are important parameters for the understanding of its ligand-binding and signalling functions.

Materials and Methods
To address these issues, we generated novel antibodies targeting TLR3, which we validated by Western Blotting, immunoprecipitation, immunofluorescence, flow cytometry, and immunohistochemistry. We evaluated by western blot TLR3 expression in 7 Non Small Cell Lung Cancer (NSCLC) and 6 Oral Squamous Cancer (OSC) cell lines from ATCC. Glycosylation steps were tracked using PNGase and endoH enzymatic degradation, and subcellular localization was evaluated by immunofluorescence and confocal microscopy. Apoptotic complex was isolated by caspase 8 immunoprecipitation.

Results
Using the novel anti-TLR3 mAbs, we observed 3 different forms of TLR3. Those forms corresponded to different maturation steps of the protein as follows: First, TLR3 is synthetized in endoplasmic reticulum (ER) in a glycosylated form (130 kda form). It transits through the Golgi where it acquires additional sugars (135 kda form). This form is very labile and is rapidly cleaved by cathepsins in acidic compartments (60 kda and 72 fragments). Despite this cleavage, the two fragments remain associated and possess a remarkably long half-life. We observed that this mature form of TLR3 was the predominant form in most cell lines studied and that the presence of an immature form was not sufficient to signal downstream of TLR3 in presence of its ligand. The mature form seems to localize to late endosomes and/or lysosomes, since it colocalized with Lysosome-Associated Membrane Protein 1(LAMP1), but not with Early Endosome Antigen 1 (EEA1).
We also found that TLR3 is associated to a bona fide Death Inducing Signaling Complex (DISC) in cancer cells, which is a property of death receptors. Importantly, no DISC was found associated with TLR3 in normal Human Bronchial Epithelial Cells (HBEpC) in presence of dsRNA.

Discussion
TLR are innate receptors belonging to a fa-

mily of pattern recognition receptors that sense the presence of pathogens and trigger a protective innate immune response. They all share structural similarities but one key characteristic that distinguishes individual TLRs is their ligand specificity.
TLR3, TLR7, TLR8 and TLR9 recognize nucleic acid and are localised in intracellular compartments (Barton & Kagan 2009). A set of new antibodies allowed us to study endogenous expression, subcellular localization, and function of TLR3, which was to date mostly studied in overexpression systems. We have shown that maturation steps resulting in a fully glycosylated and cleaved form localized in an acidic compartment are necessary for TLR3 function. Interestingly, most of the TLR3 in the cell lines we studied are present in mature form, ready to signal. These data contrast with those on TLR9, which is also cleaved and recognizes its ligand in acidic compartments. Indeed, in resting cells TLR9 is stocked in the ER and is only mobilized after ligand endocytosis (Latz et al. 2004). Moreover, whereas TLR9 cleavage releases the cytosolic fragment to form an optimal, trimmed receptor (Ewald et al. 2011), we have shown that in TLR3 both fragments remain associated. These data show that although intracellular TLRs share some structural and subcellular localisation similarities, they also possess maturation and trafficking specificities that might be linked to differences in ligand recognition and/or difference in function. Indeed, we have shown that TLR3, besides its function in detecting viral dsRNA and producing an inflammatory response, can behave like a death receptor by recruiting a DISC usually associated to death receptors, such as those of the TNF family. This recruitment seems to be specific of cancer cells, as it was not observed in normal epithelial cells, which may explain why TLR3 induces apoptosis in cancer, but not in normal cells.

Conclusions

Our study brings new data on TLR3 physiology, but information still lacking on what is the molecular switch leading to DISC formation and when during cancer cells pathogenesis this switch is operational. As cancer is a multistep process and since some oral lesion diagnosed by dentists can be attributed to a preneoplastic state, work is in progress to address these questions using oral cancer as model.

Acknowledgements

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