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Rab22a: A novel regulator of immune functions

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ABSTRACT

Dendritic cells (DCs) trigger CD8 + T cell responses after the internalization of exogenous antigens in a process called cross-presentation. Multiple intracellular transport events within the endocytic and secretory routes take place in order to accomplish this fundamental immunological process. The endomembrane system can be envisioned as a complex network of membrane domains coordinately working in the fusion of organelles, the budding of vesicles and tubules, and modifying the molecular composition of the limiting membranes. In this context of tightly regulated and dynamic endomembrane transport, small GTPases of the Rab family display a pivotal role by organizing membrane microdomains and defining specific identities to the different intracellular compartments. In this review, we synthesize and update the current knowledge about Rab22a, which has been involved in several immune functions. In this way, we analyze the intracellular localization of Rab22a and its important role in the endocytic recycling, including its relevance during MHC-I trafficking, antigen cross-presentation by DCs and the formation of T cell conjugates. We also describe how different pathogenic microorganisms hijack Rab22a functions to achieve efficient infection and intracellular survival strategies. Furthermore, we examine the oncogenic properties of Rab22a and how its expression determines the progression of many tumors. In summary, we highlight the role of Rab22a as a key effector of the intracellular trafficking that could be exploited in future therapies to modulate the immune system.

1. The logistic of antigen presentation and endomembrane organization

Antigen presentation is a complex process that requires the dynamic interaction between different organelles from the secretory and endocytic pathways. Most cells can present peptides produced in the cytosol by proteasomal digestion of endogenous proteins that are translocated into the endoplasmic reticulum (ER) to be loaded into class I molecules of the major histocompatibility complex (MHC). Once the MHC-I/peptide complex is formed, it leaves the ER and is transported to the cell surface to be presented to CD8 + T cells. Professional antigen presenting cells (APCs), such as dendritic cells (DCs), macrophages and B cells, in addition to MHC-I, assemble MHC-II molecules in the ER. This complex is transported to a specialized late endosomal compartment where is loaded with peptides produced by digestion of exogenous proteins. The MHC-II/peptide complex is transported to the plasma membrane to be presented to CD4 + T cells (Neefjes et al., 2011). Professional APCs, and in particular DCs, can also carry out cross-presentation, where exogenous antigenic material is presented in the context of MHC-I molecules. This process requires the encounter of properly processed peptides, coming from internalized material, with MHC-I molecules. Although part of the peptides can be produced in the interior of endo/phagosomes, most are generated by the transfer of proteins to the cytosol using an ER-associated machinery. Outside the endocytic pathway, proteins are cleaved by proteasomes and the peptides retrotranslocated into ER or endosomal compartments to be loaded into MHC-I by the peptide loading machinery. Finally, the MHC-I/peptide complex must traverse a secretory route to be exposed on the cell membrane and interact with CD8 + T cells (Joffre et al., 2012).

DCs are very efficient in cross-presentation and have developed a specialized endocytic pathway. The acidification of phagosomes is partially inhibited due to an incomplete activation of the V-ATPase (Trombetta et al., 2003) and the activity of the NADPH oxidase NOX2 that consumes protons (Savina et al., 2006). The slow acidification and limited access to proteases preserve intact proteins and large polypeptides for export to the cytosol. Although the molecular actors involved are still controversial, a proper cross-presentation requires that DC endo/phagosomes acquire several ER-associated proteins for transport of luminal proteins into the cytosol and translocation of proteasome-processed peptides back into endo/phagosomes by TAP1/2 transporters (Grotzke et al., 2017; Zehner et al., 2015). In this context, it has been shown that the endocytic pathway of DCs recruits ER components from

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the ER-Golgi intermediate compartment by the action of the SNARE Sec22b (Cebrian et al., 2011).

It is clear from this brief description that antigen presentation heavily depends on a sophisticated coordination of multiple intracellular transport events along the endocytic and secretory pathway and the crosstalk between these two routes. Surprisingly, the cell strategies for transport among membrane bound organelles are not completely understood. Regardless of the different opinions about the mechanism of transport, most researchers would accept that the limiting membrane of an organelle is what determines to which compartment it belongs (Pfeffer, 2017; Wandinger-Ness and Zerial, 2014). Transport can be envisioned as the changes in the limiting membrane of organelles carrying a cargo by means of i) fusion, that combines membrane domains in the same structure, ii) budding of vesicles and tubules, that permits the sorting of domains (and cargoes) in different structures, and iii) complex networks of molecular interactions that change the lipid and protein compositions of the membrane domains (Gomez-Navarro and Miller, 2016; Saimani and Kim, 2017).

2. Rab GTPases and Rab22a-mediated transport

Accepting this view of endomembrane organization and transport, Rab proteins emerge as hub factors in intracellular trafficking. It is well documented that members of the Rab family actively organize membrane microdomains (Barr, 2013; Wandinger-Ness and Zerial, 2014). Moreover, most of the 60 known members of this family present specific localization on distinct membrane-bound compartments and perturbation of Rab functions dramatically alter multiple membrane traffic steps (Diekmann et al., 2011; Mitra et al., 2011). These small GTPases act as molecular “on/off” switches cycling between inactive (GDP-bound) and active (GTP-bound) states. Rab activity is regulated by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). GEFs stimulate the exchange of GDP for GTP, generating the activated form of Rab, whereas GAPs enhance the hydrolysis of the bound GTP to GDP, inactivating Rab. Subsequently, Rab-GDP dissociates from membranes and remains in a cytosolic pool complexed with a GDP dissociation inhibitor (GDI). Membrane-associated Rabs recruit specific Rab effectors. Several of these effectors are important for membrane recognition, tethering and fusion. Others to alter the bioactive lipid composition of the membranes. Rabs also participate in the movement of organelles by regulating the association with cytoskeleton elements. The switch nature of Rabs is also an important element in the dynamics of membrane domains. Rabs can be organized in cascades fostering the “maturation” of membrane domains adding flexibility and regulation to the identity of these domains (Markgraf et al., 2007; Pfeffer, 2017; Rink et al., 2005).

Obviously, Rabs are only part of a complex molecular machinery that determines the identity, stability and dynamics of intracellular membrane domains (Barr, 2013). However, the fact that different Rabs are good markers of different intracellular compartments suggests that they play an important role in conferring identity to the membranes of the organelles. From this point of view and taken as example Rab5, a well-characterized marker for early endosomes, we can argue that this small GTPase is not recruited to early endosomes; instead, organelles carrying Rab5 domains behave as early endosomes. As a short description of the endocytic pathway, we can consider that all material incorporated by different mechanisms of endocytosis, including clathrin-dependent and independent processes are transported to Rab5 compartments. From the Rab5 organelles, cargoes can recycle back to the cell surface either via a fast recycling mode or a slower recycling route that goes through the recycling endosomes. The cargoes may also be delivered to the late endosome-lysosome system for degradation, or be transported to the *trans*-Golgi network (Mitra et al., 2011; Progida and Bakke, 2016). Each one of these transport steps requires the activity of different Rab proteins. The consensus is that Rab4 participates in the fast and Rab11 in the slow recycling pathways; Rab7 in the maturation to late endosomes/lysosomes, Rab22a in the endosomal sorting, and

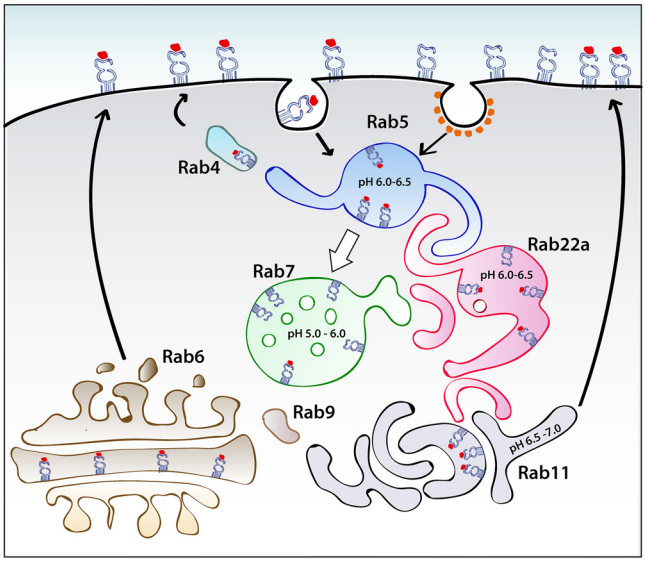

Fig. 1. Intracellular transport of MHC-I molecules along the endocytic network. MHC-I molecules are internalized by clathrin-independent endocytosis to reach Rab5-positive early endosomes. From there, they can travel back to the cell surface through a Rab4-dependent fast recycling mechanism. Also, MHC-I molecules can be transported to late endosomes by the Rab5/Rab7 switch (white arrow) or transit to Rab22a-positive sorting endosomes. In these compartments, MHC-I molecules can contact the degradative pathway by interacting with late endosomes, where most MHC-I molecules are present in an open conformation thanks to the acidic pH, or continue their transport to Rab11-positive recycling endosomes. Membrane tubulations of these compartments carry MHC-I molecules to the cell surface in a Rab11-dependent slow recycling mechanism. Additionally, loaded MHC-I molecules can access to the plasma membrane by trafficking along the secretory pathway. Interactions between the *trans*-Golgi network and the endocytic system can account through the encounter of Rab6- and Rab9-positive vesicles. The values for the pH range of early, late, sorting and recycling endosomes are depicted.

Rab9/6 in the transport to the *trans*-Golgi network. Besides membrane identity, each of these compartments is also defined by a specific range of pH that allows differential functionality, as it is exemplified in Fig. 1 for the intracellular transport of MHC-I molecules.

However, this is an oversimplification of the Rab set required for endocytosis and the list of members of the Rab family necessary for proper transport in this pathway is quite larger. The case of Rab22a is interesting to highlight for several reasons. This small GTPase has not been so rigorously characterized, as compared to other Rab proteins. Nevertheless, recent implications of Rab22a in different immune functions center this molecule as a key factor to better understand some immunological processes that involve complex endocytic interactions. Then, more and more Rab22a is being shown to participate in a large number of infection scenarios by controlling different pathogen’s trafficking events.

So far, what we do know about Rab22a is that is required for sorting and recycling of molecules internalized by clathrin-dependent and independent mechanisms. Rab22a is closely related to Rab5 and share effector with Rab5 and Rab4 (Diekmann et al., 2011; Eathiraj et al., 2005; Li and Yu, 2016). It binds EEA1 (Kauppi et al., 2002) and recruits Rabex-5, a GEF for Rab5 (Zhu et al., 2009). In CHO cells it has been shown that overexpression of Rab22a wild type and a constructively active mutant strongly affect the morphology and function of early endosomes (Mesa et al., 2001). In these experiments, the transport of rhodamine-dextran to lysosomes or the digestion of internalized proteins was not affected. In contrast, the retrograde transport of cholera toxin from endosomes to the Golgi apparatus was strongly delayed (Mesa et al., 2005). Kinetic analysis indicates that transferrin internalization, a clathrin-dependent process, and fast recycling were not

affected by Rab22a expression. The transferrin receptor was redistributed to large Rab22a-positive structures in the cell periphery and transferrin recycling from these compartments was inhibited. In Hela cells, Rab22a depletion by small interfering RNA disorganized the perinuclear recycling center and also affected transferrin recycling (Magadan et al., 2006). In these cells, Rab22a associates with tubular recycling structures containing proteins internalized by clathrin-independent mechanisms (Barral et al., 2008; Weigert et al., 2004). The depletion of Rab22a strongly inhibits the recycling of these proteins. In summary, results from several laboratories indicate that Rab22a membrane domains participate in the recruitment of a subset of cargo proteins that are recycled to the cell surface by specialized pathways.

Interestingly, many proteins that are internalized by clathrin-independent mechanisms and recycle by a Rab22a-dependent pathway, are part of the adaptive immune response (Johnson et al., 2017). However, the role of Rab22a during the intracellular transport of these proteins has been studied in non-specialized cells. Only recently the function of Rab22a has been analyzed in cells of the immune system. In 2016, we showed that Rab22a displays a pivotal role during MHC-I intracellular trafficking and antigen cross-presentation by DCs (Cebrian et al., 2016). We demonstrated that Rab22a stabilizes the intracellular pool of MHC-I, regulates the delivery of these molecules to phagosomes and the recycling to the cell surface. All these defects observed in Rab22a knock-down DCs significantly impacted on their ability to cross-present exogenous antigens, but not in their capacity to internalize soluble or particulate antigens. Furthermore, endogenous MHC-I presentation was not altered by the silencing of Rab22a in DCs, indicating that ER to Golgi trafficking is fully functional in these cells (Cebrian et al., 2016). More recently, we also showed in DCs that Rab22a does not interfere with the normal recruitment of ER-derived proteins to phagosomes, but it does regulate the acquisition of these same molecules to endosomes. Accordantly, early endosomal maturation is drastically impaired in Rab22a-deficient DCs, but the maturation of latex bead phagosomes seems normal in these cells (Croce et al., 2017). Nevertheless, the relevance of Rab22a during the maturation process of different pathogens-containing phagosomes is quite different, as it is described below. Altogether these data suggest that Rab22a display key functions throughout the endocytic network of DCs. Moreover, it was recently published by the group of Julie Donaldson that Rab22a and Arf6 activities drive the formation of T cell conjugates through a clathrin-independent endocytosis. In this study, the authors observed that upon lymphocyte activation Rab22a polarizes to the sites of contact with APCs, and that overexpression of the GTP-binding defective mutant Rab22S19N (dominant negative form of Rab22a) inhibited MHC-I internalization, T cell conjugate formation and cell spreading (Johnson et al., 2017). Hopefully, future studies involving different immune cell types and *in vivo* animal models will help to expand and understand the increasing panel of effector functions that Rab22a displays during the activation of the immune system.

3. Role of Rab22a during pathogens' trafficking

Most intracellular pathogens survive and replicate within specific organelles adopting different strategies in order to take control of critical membrane trafficking events. For this, they have evolved several mechanisms to target and hijack, preferentially, key Rab GTPases that modulate endosomal transport, such as Rab5, Rab7 or Rab14. Moreover, sorting and recycling endosomes appear as important intracellular platforms to guarantee the success of transit inside eukaryotic cells for many pathogens. In this context, Rab11 has been the most studied target in the past years, but also Rab4 and Rab22a (and their effectors) emerge as relevant molecules during the establishment of a wide spectrum of infections. As we have previously discussed, for the particular case of Rab22a is important to notice that it controls critical steps of CD1a and MHC-I intracellular trafficking. So, we consider of high interest to summarize the current knowledge about Rab22a in the context of pathogenic infections.

The first evidence that a microorganism was able to recruit Rab22a and inhibit phagosomal maturation appeared a decade ago with a seminal study involving the intracellular pathogen *Mycobacterium tuberculosis* (Roberts et al., 2006). Some years before, the same laboratory had shown that upon infection, phagosomal maturation is arrested and phagolysosome biogenesis impaired because mycobacterial phagosomes are not able to undergo Rab5-Rab7 conversion (Via et al., 1997). But in the work of Roberts and colleagues, they clearly demonstrated in infected macrophages that Rab22a is actively recruited to mycobacterial phagosomes to block Rab7 acquisition to these organelles and prevents phagosomal maturation, positioning Rab22a as a critical checkpoint for Rab7 conversion (Roberts et al., 2006).

Four years later, a report with the obligate intracellular bacterium *Anaplasma phagocytophilum*, which infects granulocytes and bone marrow progenitor cells, showed that this microbe also intercepts Rab22a-positive recycling vesicles (Huang et al., 2010). In the same line as *M. tuberculosis*, the *A. phagocytophilum*-occupied vacuole (ApV) is highly adapted to avoid acidification, lysosomal fusion and proteolytic degradation (Carlyon et al., 2004; Mott et al., 2002; IJdo and Mueller, 2004). In this context, classical phagosomal maturation and fusogenicity processes are drastically altered, although MHC-I and II molecules are present in the ApV (Mott et al., 1999). By screening a panel of 20 fluorescent protein-tagged Rab GTPases, Huang and colleagues showed that the ApV selectively recruits recycling endosomes in a tetracycline-sensitive manner, suggesting a dependence of bacterial protein synthesis. With the exception of Rab1, that localizes at the ER exit sites and mediates ER to Golgi trafficking, the authors found that Rab4a, Rab10, Rab11a, Rab14, Rab22a and Rab35, all key regulators of endocytic recycling, associate with the ApV in HL-60 infected cells (Huang et al., 2010).

Many reports pointing towards Rab22a as a major endosomal target during pathogen infection were done with *Legionella pneumophila*, a Gram-negative bacterium that replicates in a highly specialized vacuole (*Legionella*-containing vesicle, LCV) inside lung macrophages (Isberg et al., 2009). Similarly as the ApV, the LCV avoids lysosomal fusion (Horwitz, 1983), but it disposes of the Dot/Icm type IV secretion system to translocate more than 300 bacterial effectors into the cytosol and hence to hijack many host cell functions and intracellular trafficking events (Zhu et al., 2011; Hubber and Roy, 2010). In 2012, Ku and colleagues described how the C-terminal domain of the bacterial effector VipD (vacuolar protein sorting inhibitor protein D) establishes tight interactions with the GTP-bound active forms of Rab5 and Rab22a blocking endosomal trafficking and lysosomal degradation in mouse macrophages (Ku et al., 2012). Two years later, an independent study from a different research group also focused on VipD, found that this bacterial effector exhibits phospholipase A_1 activity upon the binding to Rab5 or Rab22a. These interactions trigger phospholipase A_1 catalytic activity specifically, altering the composition of endosomes by phosphatidylinositol-3-phosphate removal from the endosomal membranes and rendering LCVs non-fusogenic (Gaspar and Machner, 2014). Moreover, a recently identified *L. pneumophila* effector called Lpg0393 was shown to share structural similarities with the catalytic core of Rabex-5. Then, functioning as a bacterial GEF, Lpg0393 activates Rab5, Rab21 and Rab22a (Sohn et al., 2015).

Another Gram-negative bacterium, *Neisseria meningitidis*, a diplococcus that colonizes the upper respiratory tract and must translocate the mucosal barrier in order to accomplish dissemination to different organs, was also shown to intercept Rab22a function. In this study, Barrile and colleagues demonstrated that *N. meningitidis* alters plasma membrane composition, and subverts intracellular transport and host cell architecture to cross the nasopharyngeal barrier (Barrile et al., 2015). This barrier consist of highly polarized epithelial cells, which have a complex intracellular transport machinery specialized to deliver membrane-associated molecules either to basolateral or apical domains to maintain normal cell polarity and the integrity of epithelial monolayers (Fölsch, 2008). The authors showed that *N. meningitidis* alters

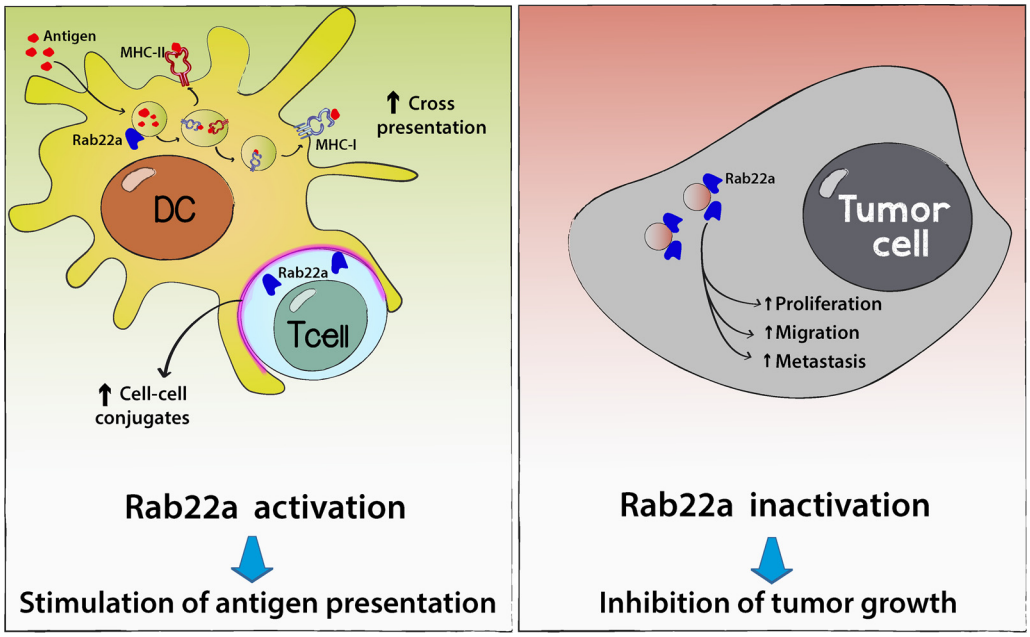

Fig. 2. Rab22a is a determinant molecular effector of different immune functions. In DCs and T lymphocytes Rab22a activity assures the proper intracellular trafficking of MHC-I molecules. In DCs this guarantees an efficient antigen cross-presentation process and in T lymphocytes the polarization of Rab22a towards the immunological synapse facilitates the formation of T cell conjugates. Envisioning a novel therapeutic strategy, the enhancement of Rab22a activity in DCs and T lymphocytes could stimulate antigen presentation (left panel). In tumor cells, Rab22a expression induces cell proliferation, migration and metastasis, favoring tumor growth. So, in contrast to the previous situation, the therapeutic strategy in the tumor microenvironment would consist in persuading Rab22a inactivation in order to inhibit tumor growth and metastasis (right panel).

epithelial cell polarity without disrupting its integrity by recruiting Rab11, Rab22a and Rab3-positive recycling vesicles to the apical site of infection. This interaction between *N. meningitidis* and recycling endosomes induces the recruitment of different basolateral-soluble factors to the bacterial colonies in a microtubule-dependent mechanism to overcome the epithelial barrier and achieve systemic dissemination (Barrile et al., 2015).

Furthermore, it was shown that Rab22a plays a role in the vaccinia virus intracellular transport (Hsiao et al., 2015). This virus belongs to the family *Poxviridae* and is able to infect many different cell types. After viral internalization, mature virus-containing vesicles locate into CD98 and phosphatidylinositol-3-phosphate-positive macropinososmes that then fuse with Rab5-positive endosomes (Schroeder et al., 2012). In this work, Hsiao and colleagues described in HeLa cells by single-particle tracking how vaccinia virus recruits WASH-VPEF/FAM21-retromer complexes after the transit to Rab5 early endosomes. In addition, the authors showed that these complexes perform endosomal membrane fission and cargo sorting to Rab11 and Rab22a-positive recycling endosomes where the virus fuses with the limiting membrane of the organelles and is released into the cytoplasm (Hsiao et al., 2015). In a more recent study, Karleuša and colleagues showed that in the early phase of murine cytomegalovirus (MCMV) infection, the endosomal trafficking of TfR, MHC-I (recycling compartments), EGFR, M6PR and Lamp1 (late endosomes) is significantly altered (Karleuša et al., 2017). MCMV, as other members of the family *Herpesviridae*, provokes strong rearrangements of the host cell endomembrane system (Johnson and Baines, 2011). In this work, the authors demonstrated that MCMV infection perturbs the endocytic transport at the level of sorting endosomes and endosomal recycling compartments by monitoring the accumulation of internalized cargo within Arf6-, Rab5-, Rab22a- and Rab11a-positive structures. This rearrangement of the endosomal system takes place very soon after infection, suggesting that may be important for the initial steps of the virus assembly compartment biogenesis (Karleuša et al., 2017).

Interestingly, in 2015 Naj and Linder published that the uptake and processing of the spirochete *Borrelia burgdorferi*, the causative agent of Lyme disease, is regulated by Rab22a and Rab5a (Naj and Linder, 2015). In this study, the authors first showed that borreliae-containing phagosomes positive for Rab22a were found in close proximity to Rab5a vesicles and that *B. burgdorferi* internalization was accompanied by phagosomal surface reduction and spirochete compaction. Moreover, they demonstrated that ER tubules facilitate the contacts between Rab22a- and Rab5a-positive endosomes during *B. burgdorferi* compaction. Finally, the authors described how the activities of these two Rab GTPases are essential to guarantee proper phagosomal maturation and efficient lysosomal degradation of *B. burgdorferi* by primary human macrophages (Naj and Linder, 2015). This report supports the increasing evidence for Rab5 and Rab22a as key regulators of microbe infection and intracellular trafficking, and triggers the idea for a possible direct connection between the ER and Rab22a-positive compartments.

More recently, we have shown that the causative agent of toxoplasmosis, the obligate intracellular parasite *Toxoplasma gondii*, strongly recruits Rab22a-positive vesicles to the parasitophorous vacuole (PV) after DC infection (Cebrian et al., 2016). The PV is a highly specialized compartment distinct from classical phagosomes and surrounded by a limiting membrane that restricts access to endo/lysosomes, but it actively recruits ER and mitochondria (Goldszmid et al., 2009; Melo and de Souza, 1997; Sinai et al., 1997). As the PV grows, it deeply impacts on the microtubule cytoskeleton that reorganizes around the vacuole (Martin et al., 2007). Although multiple interactions between the PV and host organelles have been described, the molecular effectors driving these processes and the functional consequences for parasite growth and/or host defense remain largely unknown. In that study, we showed that *T. gondii*-associated antigens are not presented properly to CD8 + T lymphocytes in Rab22a KD DCs. Furthermore, we showed for the first time the interception of recycling compartments by the PV, as labeled for Rab22a, upon *T. gondii* active DC infections (Cebrian et al., 2016). Ongoing studies in our group will help us to define the relevance of these interactions with host recycling vesicles for the parasite fitness.

4. Rab22a expression in tumors

Changes in gene expression of several small GTPases involved in vesicular transport during carcinogenesis was reported fifteen years ago, and RAB22A was found to reside in regions of chromosomal breakpoints (He et al., 2002). More recently, RAB22A was identified as a relevant target gene of microRNAs (miRNA, non-coding RNAs that negatively regulate gene expression at the post-transcriptional level) in several tumor models. This point towards RAB22A as a critical oncogene and show that the overexpression of this small GTPase in miRNAs downregulation-dependent mechanisms favors tumor growth and carcinogenesis.

Two recent reports bring some clarification on the mechanistic bases of Rab22a during carcinogenesis and metastasis. The first one, is a

study that used an hypoxia-induced model and it showed that extracellular microvesicles shedding by breast cancer cells, which promotes focal adhesion formation, invasion and metastasis, depends on RAB22A expression (Wang et al., 2014). In the second study, Zhou and colleagues found that Rab22a regulates lung cancer cell migration and invasion through the recycling of the extracellular matrix metalloproteinase inducer (EMMPRIN), also known as CD147 (Zhou et al., 2017).

Putting all this information together, and taking into consideration that the cross-presentation of tumor-derived antigens is essential to elicit efficient CD8 + T cell responses and anti-tumor immunity, we consider that Rab22a could play a major role for the design of novel immunotherapy-based approaches. Ideally, the most optimal therapeutic situation would consist of enhancing Rab22a activity selectively in DCs and lymphocytes in order to stimulate antigen presentation, but blocking at the same time either Rab22a expression or activity in cancer cells to avoid tumor growth and metastasis (Fig. 2).

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