Rho GTPases signalling in cancer development and metastasis

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Rho GTPases are a family of small signalling G proteins, belonging to the Ras superfamily. They function as GDP/GTP-related molecular switches cycling between active, GTP- bound, and inactive, GDP bound, states. The activation of GTPases is stimulated by guanine nucleotide exchange factors (GEFs), while GTPases-activating proteins (GAPs) promote their inactivation (Figure 1) [1]. Rho family members can be activated by various extracellular stimuli and, once activated, interact with cellular target proteins and effectors, triggering a wide number of cellular responses. The main function of Rho proteins is to control the actin cytoskeleton, thus, they drive many essential cellular processes, such as morphogenesis, endocytosis, migration and cytokinesis [2]. Rac (1, 2, 3), Cdc42 and Rho (A, B, C) are the best characterised members of this family. This review will focus on the biological role of Rho GTPases in the cell and their role in human cancer, before considering their potential as drug targets.

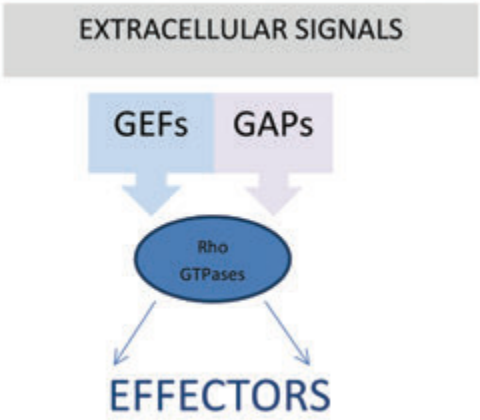

Figure 1. Rho GTPases cycle between active states, promoted by GEFs, and inactive states, occurring after GAP binding. When active, Rho proteins bind and activate their effectors, initiating a signalling cascade.

Biological role of Rho GTPases in cell signalling

The main function of Rho GTPases is to control the assembly and the disassembly of actin filament and the reorganisation of actin cytoskeleton, processes that must be coordinated for a cell to migrate. Cell migration occurs in different steps. Firstly, a protusion is generated from the cell, an adhesion site is established at the front, the cell contracts and, finally, detaches from the adhesion point at its rear [3]. It is assumed that each Rho GTPase is central to different signalling pathways controlling migration, but currently just Rac (1, 2, 3), Rho (A, B, C) and Cdc42 have been studied in detail [4]. The three Rho isoforms are responsible for the cross-link of myosin and actin filaments and the generation of contractile force, resulted by Rho interaction with mDia and p160 Rho kinase. Rac proteins and Cdc42 both activate the Actin Related Protein 2/3 complex (Arp 2/3), but through different mediators. Rac (1, 2, 3) interact with the specifically Rac1-associated protein 1 (Sra-1), whereas Cdc42 effector protein is Wiskott-Aldrich syndrome protein (WASP). As a result, Rac pathway leads to the formation of lamellipodia and the Cdc42 cascade causes the generation of filopodia [4]. Cdc42 is also significant in the establishment and the maintenance of anterior-posterior and apical-basal cell polarity [4], through its interaction with partitioning-defective protein 6 (Par6), Par3 and atypical protein kinase C (aPKC) [5]. Thus, in the migration process, Cdc42 is essential for the direction of movement, Rac (1, 2, 3) for the protusions and Rho for contraction [3]. These proteins have also been reported to play a role in cell adhesion. Rho (A, B, C) regulate integrin-mediated focal adhesion interaction with Rho-kinase, while Cdc42 and the three Rac isoforms influence cadherin-mediated cell-cell adhesion, inhibiting IQGAP1 [6]. The signalling cascades in which the other Rho GTPases are involved are not fully understood and further research could provide a complete explanation of cell migration and other processes driven by actin organisation, such as morphogenesis, endocytosis and cytokinesis. Because of their essential role in migration and polarity, Rho GTPases are particularly significant in organ development and embryogenesis [4].

Role of Rho GTPases in human cancer

Since they influence many cellular processes which may affect cancer progression, such as cell cycle, gene transcription, cell survival, cell migration and vesicle transport, it is not surprising that deregulation of Rho GTPases

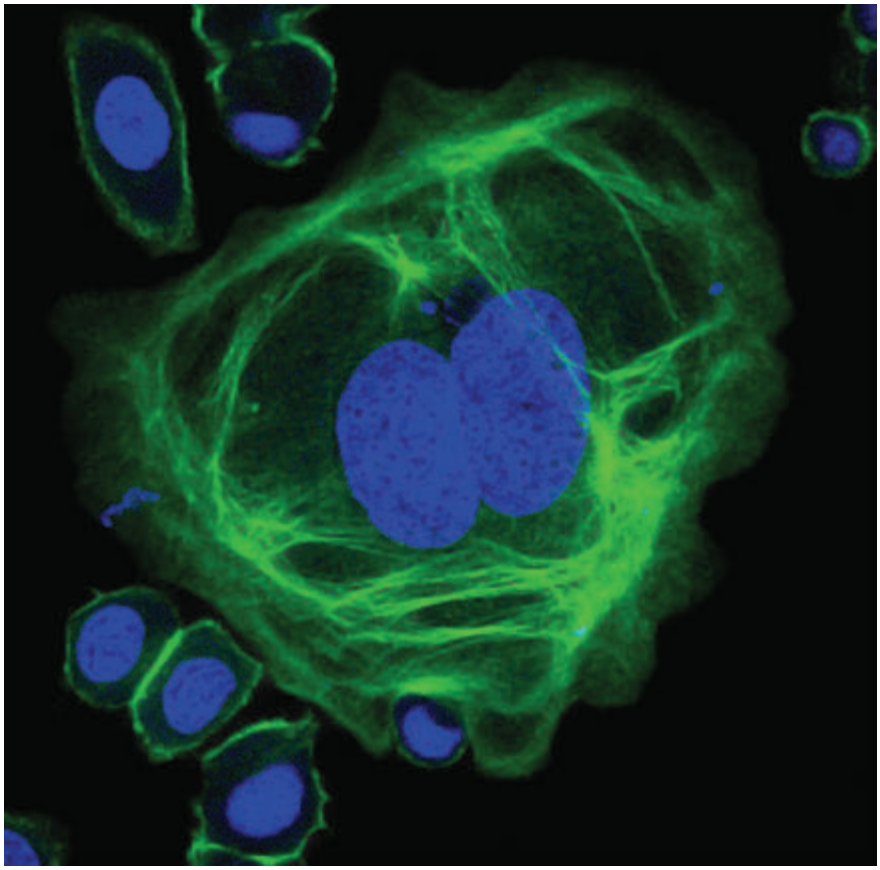

Figure 2. Confocal image of p185HER-2 -overexpressing SK-BR-3 cells. Cells incubated with nuclear stain TO-PRO3 (blue) and actin label Phalloidin-FITC conjugate (green). Image shows formation of very large multinucleated cells and deregulation of an actin cytoskeleton.

promotes abnormal cell proliferation (Fig. 2) and have a significant role in cancer development and metastasis [2]. However, the functional role of GTPases in human cancer has been elucidated in just a few scenarios. For instance, high proteins levels of RhoA have been observed in hepatocellular, melanoma, colorectal, ovarian and bladder cancers and this protein has been reported to be involved in cancer proliferation and survival, but also invasion and migration [2]. Similarly, high proteins levels of Rac1 have been suggested to drive cancer proliferation in testicular, gastric and breast cancer [7]. While these proteins drive cells growth, high protein levels of RhoC and overexpression of Cdc42 have been reported to induce cell invasion and migration resulting in cancer metastasis in various tumours [7, 8]. The role of other Rho family members in human cancer is not fully understood yet, but each of these proteins has been observed to have an altered expression and/or to be mutated in human cancer [2]. Further investigation is needed into the molecular signalling that occurs during tumour development and progression, in order to identify the proteins involved in this process and their function in physiology and disease. There is potential to use GTPases as molecular markers in clinical practice and as alternative therapeutic targets for cancer.

Current knowledge and future perspectives of GTPases as a cancer drug targets

Whilst the current knowledge surrounding the function of Rho proteins is fairly limited, a recent article illustrated the potential for the clinical use of an anti-GTPase. Zins and his group demonstrated the effect of a small molecule drug, AZA197, in human colon cancer cells, first in vitro, and then in vivo, using a xenograft mouse model of human colon cancer. AZA197 has been demonstrated to be specific for Cdc42, to suppress cell proliferation, migration and invasion and to increase apoptosis [9]. The data showed the potential of a cancer therapy that is directed at the Rho GTPases. Moreover, since Rho proteins can endow cancer cells with elevated metastatic ability and since the development of metastasis are the cause of 90% of cancer-related mortality [10], further investigation in the intracellular pathways and design of new therapeutic compounds could be of great importance for the treatment of cancer. In conclusion, continued investigation into the function of Rho proteins is necessary in order to develop new drugs to further impact on patient survival.

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