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Signalling networks in focus

Rho GTPases and p21-activated kinase in the regulation of proliferation and apoptosis by gastrins

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ABSTRACT

Gastrins, including amidated gastrin (Gamide) and glycine-extended gastrin (Ggly), accelerate the growth of gastrointestinal cancer cells by stimulation of proliferation and inhibition of apoptosis. Gamide and Ggly activate different G proteins of the Rho family of small GTPases. For example, Gamide signals Rac/Cdc42 to activate p21-activated kinase 1 while Ggly signals Rho to activate Rho-activated kinase. p21-activated kinase 1 and Rho-activated kinase induce changes in phosphorylation or expression, respectively, of proteins of the Bcl-2 family, which then affect the caspase cascade with consequent inhibition of apoptosis. In addition, interaction of p21-activated kinase 1 with β-catenin results in phosphorylation of β-catenin, which enhances its translocation in to the nucleus, activation of TCF4-dependent transcription, and proliferation and migration. The central role of the β-catenin pathway in carcinogenesis suggests that specific inhibitors of p21-activated kinase 1 may in the future provide novel therapies for gastrointestinal malignancies.

1. Introduction

The classical gastrointestinal hormone gastrin was first described as a stimulant of gastric acid secretion in 1905. Recent interest has focussed on the role of gastrins as growth factors for the normal gastrointestinal mucosa and in the development of gastrointestinal cancer (Aly et al., 2004). Interestingly different forms of gastrins act via different receptors located in different regions of the gastrointestinal tract. All forms of gastrin are derived from a 101 amino acid precursor, preprogastrin. After removal of the N-terminal signal peptide, endo- and carboxypeptidase cleavages yield glycine-extended gastrin (Ggly), C-terminal amidation of which generates mature amidated gastrin (Gamide).

2. Functions

The effects of Gamide are more marked in the upper gastrointestinal tract while Ggly is more potent in the colon. Gamide acts as a growth factor for the normal stomach and pancreas, and also stimulates the growth of malignant gastrointestinal cells *in vitro* and as xenografts in mice (reviewed by Ferrand and Wang, 2006). Ggly is also biologically active and, like Gamide, promotes the growth of several cell types, including human and mouse colorectal cancer (CRC) cells. In animal models, progastrin and Ggly stimulate proliferation of the normal colonic mucosa, and increase the number of CRC after treatment with the carcinogen azoxymethane (Ferrand and Wang, 2006). Over the last decade, a role for Gamide has been demonstrated in adhesion and migration, and Ggly and progastrin have recently been shown to modulate the subcellular localization of adhesion proteins and to promote cell migration in wound healing assays (Hollande et al., 2001).

The biological actions of Gamide are mediated by the cholecystokinin receptor type 2 (CCK2R), a member of the G protein-coupled receptor family. Gamide, acting via the

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Signalling network facts

- Gastrins accelerate the growth of gastrointestinal cancer cells *in vitro* and *in vivo*.
- The Rho family of small GTPases act as molecular switches which control multiple cellular processes. Gamide and Ggly signal different Rho GTPases to activate p21-activated kinase 1 (PAK1) and Rho-activated kinase (ROCK), respectively.
- PAK1 and ROCK are serine/threonine kinases. PAK1 in particular functions as a node for several signalling pathways, and is essential for both Gamide- and Ggly-induced inhibition of apoptosis, and stimulation of proliferation and migration.
- By activation of PAK1, gastrins stimulate phosphorylation of β-catenin and induce a shift of β-catenin from the cell membrane to the nucleus, where β-catenin activates TCF4-dependent transcription and enhances proliferation and migration.

CCK2R, activates several signalling pathways linked to proliferation, migration and anti-apoptotic effects (reviewed by Ferrand and Wang, 2006; Grabowska and Watson, 2007). For example, Gamide stimulates phosphatidylinositol 3-kinase (PI3-K) /Akt-dependent pathways, activates JNK and p38 MAPK through a PKC-dependent pathway, and induces the phosphorylation of the adaptor protein Shc, which associates with the complex Grb2/Sos, leading to activation of the Ras/Raf/MEK/ERK cascade (Todisco et al., 2001; Ferrand and Wang, 2006). Inhibition of gastrin gene expression by small interfering RNA (Grabowska et al., 2007) or short hairpin RNA (Pannequin et al., 2007) reduces growth and promotes apoptosis of gastrointestinal cancer cells. Gamide inhibits apoptosis through regulation of proteins of the Bcl-2 family, and proteases of the caspase family (Konturek et al., 2003).

Despite the similarity in structure between Ggly and Gamide, the biological actions of Ggly are not mediated by the CCK2R, and the structure of the receptor for Ggly remains unknown. Like Gamide, Ggly promotes cell proliferation and inhibits apoptosis by activation of PI3-K/Akt- and JNK-dependent pathways (Hollande et al., 2001; Beales and Ogunwobi, 2006).

3. Cascades

3.1. Rho-GTPases and PAK1

Activation of the Rho family of small GTPases (Rho, Rac and Cdc42) from the inactive GDP-bound form to the active GTP-bound form has been implicated in complex biological processes, such as growth, survival and apoptosis (Jaffe and Hall, 2005). Rho acts through interaction with specific target proteins, such as the serine/threonine protein kinase Rho-activated kinase (ROCK). Like Rho, Rac and Cdc42 also act through specific effector proteins, such as the p21-activated kinases (PAKs). PAK1, the best-characterized member of the PAK family, was originally identified as a protein that interacts with Rac and Cdc42. PAK1 mediates growth factor-stimulated cell growth either by promoting proliferation via the mitogen-activated protein kinase (MAPK)-dependent pathway or by inhibiting apoptosis by phosphorylation and inactivation of Bad, a pro-apoptotic member of the Bcl-2 family of proteins, and by activation of nuclear factor-kappa B (Bokoch, 2003).

A large body of experimental evidence links PAK1 to cellular mechanisms important for cellular transformation and tumour progression. PAK1 signalling not only serves as a convergence point in transformation induced by Rho GTPases that are activated by mitogenic factors (Kumar et al., 2006), but also is required for VEGF expression and VEGF-induced angiogenesis (Bagheri-Yarmand et al., 2000), which in turn promotes tumour growth and metastasis. In addition to its role in the cytoplasm, PAK1 also has a well-established role in the nucleus (Kumar et al., 2006), where it associates with chromatin leading to modulation of transcription. In particular, PAK1 phosphorylates Snail and promotes transcriptional repression by Snail (Kumar et al., 2006). The resultant decrease in the expression of E-cadherin contributes to the epithelial-to-mesenchymal transition, a change in cellular phenotype associated with the progression of cancers of epithelial origin (Thiery, 2002). Alterations in PAK1 expression and activation have been detected in human tumours. In CRC, PAK1 expression was found to increase with progression through the adenoma to carcinoma sequence, with the most dramatic increases in invasive and metastatic CRC (Carter et al., 2004).

3.2. Bcl-2 proteins, caspases, and apoptosis

Apoptosis, or programmed cell death, occurs in all cells by highly efficient mechanisms. The family of Bcl-2 proteins, a group of crucial regulatory factors in apoptosis, contains both pro-apoptotic (Bax and Bad) and anti-apoptotic (Bcl-2 and Bcl-xl) members. Pro-apoptotic Bcl-2-like proteins are further divided into BH3-domain only (Bad) and multi-domain (Bax) members. The anti-apoptotic Bcl-2-like proteins form heterodimers with Bax or Bad, and inhibit their pro-apoptotic effect. Hence the ratio of pro-apoptotic to anti-apoptotic Bcl-2-like proteins dictates the susceptibility of cells to an apoptotic stimulus (reviewed by Cory and Adams, 2002). Interaction between proapoptotic proteins of the Bcl-2 family results in release of cytochrome *C* from mitochondria, with consequent activation of the caspase cascade. Caspases, a set of cysteine proteases, are activated specifically in apoptotic cells, and are recognized as the central executioners of the apoptotic pathway as their activation brings about most of the changes that characterize cell apoptosis. The effector caspase 3, for example, has been shown to be a critical mediator of apoptosis initiated by mitochondria (Cory and Adams, 2002).

The Rho family of G proteins plays an important role in apoptosis. Activation of Rho prevents apoptosis by increasing expression of the anti-apoptotic proteins Bcl-2 and Bcl-xl (reviewed by Bokoch, 2000). Inhibition of Rac triggers cell apoptosis associated with increased activation of the pro-apoptotic protein Bax and expression of another pro-apoptotic protein Bim, and activation of caspases 9 and 3. Inhibition of ROCK induces apoptosis through