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Laboratory-Clinic Interface

RAF signaling in neuroendocrine neoplasms: From bench to bedside

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ABSTRACT

Neuroendocrine neoplasms are a low-incidence and heterogeneous group of malignancies. In the advanced stage, several therapeutic options can be discussed, including molecular-targeted agents, but biological predicting factors are lacking. A number of molecular targets have been studied over the last decade leading to several phase II studies; however, very few agents progressed to phase III clinical trials. The RAF family of proteins belongs to the mitogen-activated protein kinase (MAPK) pathway, that has a role in several types of cancers, particularly related to BRAF mutations. Indeed BRAF inhibitors have been reported as being effective, mainly in melanoma. However, in neuroendocrine neoplasms BRAF mutations are extremely rare and RAF-1 activation has been reported to inhibit tumor growth in a pre-clinical setting. Therefore, in this field, RAF-1 activators rather than BRAF inhibitors should be clinically investigated. This article reviews the basic science as well as clinical data of RAF signaling in advanced neuroendocrine neoplasms with special emphasis on the potential role of both RAF activators and inhibitors.

Introduction

Neuroendocrine neoplasms (NENs) derive from the diffuse neuroendocrine system [1]. They represent a group of low-incidence and heterogeneous neoplasms that can arise in almost all organs of the body [2]. In two thirds of cases, they arise from the gastroenteropancreatic (GEP) tract, most commonly from small bowel (30%) and pancreas (16%) [3]. Gastroenteropancreatic NENs are classified according to Ki-67 label index and/or mitotic index (MI) in G1 (Ki-67 $\leqslant$ 2% and/or MI < 2), G2 (Ki-67 3–20% and/or MI 2–20), and G3 (Ki-67 > 20% and/or MI > 20) [4].

Clinically, these neoplasms are classified as functioning or non-functioning, based on the presence or absence of clinical manifestations related to excess hormone production [5].

Treatment of advanced NENs is various, including several different types of systemic therapies, such as chemotherapy, somatostatin analog (SSA), interferon (IFN), peptide radio-receptor therapy (PRRT), and molecular targeted agents (MTAs). Unfortunately, clinical and/or biological predicting factors for response/efficacy are lacking. Over the last decade, a number of disregulated genes related to the pathogenesis and progression of NENs have been identified, some of them also representing potentially druggable molecular targets [6]. The mTOR pathway has been demonstrated to have an important role in the development of NENs, particularly from pancreas (PNENs) [7,8]. The mTOR inhibitor everolimus has been largely studied in NENs and it has been approved by Food and Drug Administration (FDA) and European Medical Agency (EMA) for patients with advanced well/moderately differentiated progressing PNENs. In the same context and time, the multi-target inhibitor sunitinib has been approved as well. Many other MTAs are currently under investigation in NENs.

In this review, we will provide an overview of the pre-clinical and clinical data relating to RAF (rapidly accelerated fibrosarcoma) signaling as a potential target of treatment in NENs.

Biology of MAP kinase pathway

The mitogen-activated protein kinase (MAPK) signaling pathway plays a fundamental role in coordinating gene expression in eukaryotes and connects extracellular signals to the intracellular machinery that controls important cellular processes like growth,

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proliferation, differentiation and migration [9,10]. So far, four different MAPK cascades have been described, named after the individual MAPK component: extracellular signal–regulated kinase (ERK), c-Jun N-terminal kinase (JNK), p38 and ERK5/Big MAPK (BMK) [11].

The ERK pathway is the best described and it is the paradigm for the MAPK modules in general. Based on the three-tiered kinase module, downstream the growth factor receptors, the ERK cascade is initiated by RAS, that activates the MAP3K (MAP kinase kinase kinase) RAF, recruiting it from the cytosol to the cell membrane. Then RAF activates the MAP2K mitogen-activated protein kinase/extracellular signal-regulated kinase (MEK), which in turn phosphorylates and activates ERK [11] Fig. 1.

The ERK pathway is deregulated in about one third of all human cancers, playing a significant role in cell proliferation and transmitting signals that promote both growth and differentiation. A large body of evidence indicates that over-activation of the ERK pathway is involved in the pathogenesis and progression of human cancer including breast cancer, non–small cell lung cancer, head and neck squamous cell carcinoma, as well as colorectal carcinoma [12–14]. Moreover, inhibition of RAS and RAF in cell lines with activating RAS mutations leads to sensitization to chemotherapy and ionizing radiation [15].

Following activation of the ERK signaling pathway, transcription factors are phosphorylated and then activated, leading to target genes expression and resulting in biological responses [16]. Changes in ERK pathway regulation resulting from either mutations or changes in the expression of proteins regulating the process of ERK signaling, such as epidermal growth factor receptor (EGFR) mutations or mutations of RAS or RAF, can contribute to cancer, as well as other diseases [17]. RAS is the oncogene most commonly mutated in human cancers (around one third of cases). Three human RAS genes are known, including H-RAS, K-RAS (Harvey and Kirsten strains of the mouse sarcoma virus, respectively), and N-RAS (neuroblastoma RAS viral oncogene homolog) [18] Fig. 2.

RAF family of proteins as a key player in carcinogenesis

The RAF family of proteins (including RAF-1/CRAF, ARAF, BRAF) are serine/threonine protein kinases that are activated by RAS. As a result of RAS activation and based on cellular context, signaling may proceed through the formation of BRAF–CRAF dimers [19,20]

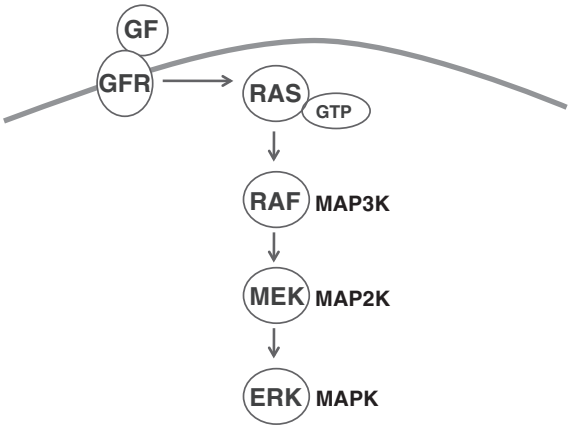

Fig. 1. The three-tiered kinase module ERK pathway. GF, growth factor; GFR, growth factor receptor; RAS, Rat sarcoma; GTP, guanosine triphosphate; RAF, rapidly accelerated fibrosarcoma; MAP3K, mitogen activated protein 3 kinase; MEK, MAPK/extracellular signal-regulated kinase; ERK, extracellular signal-regulated kinase; MAP2K, mitogen activated protein 2 kinase; MAPK, mitogen activated protein kinase.

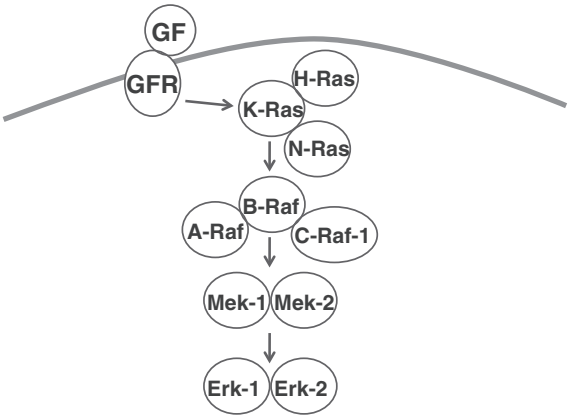

Fig. 2. Schematic representation of the ERK signaling pathway illustrating the different isoforms of MAPK pathway components. Binding of a growth factor to the membrane receptor first activates Ras by phosphorylation. This leads to a sequential phosphorylation of downstream effectors Raf/MEK/ERK. This leads to the consequent activation of transcription factors, which in turn coordinates cellular proliferation and apoptosis.

(Fig. 2). However, mutant forms of BRAF, including the V600E, are constitutively active and thus capable of phosphorylating MEK in a RAS-independent manner [21].

Most RAF functions are believed to be conducted through phosphorylation and consequently activation of the MEK1 and MEK2 [22,23]. Activated MEK1/2 then phosphorylate and activate ERK1 and ERK2, which in turn regulate important cellular functions [24].

Although the RAF family proteins share many biological features, there are however important differences. These differences provide a biological basis for the fact that a mutation at codon 600 leads to constitutive BRAF activation, whereas the similar mutation in CRAF does not [25]. They also supply a possible explanation for the important question of why BRAF is by far the most commonly mutated RAF protein in human cancer [26].

The $BRAF^{V600E}$ represents the most common (90%) RAF mutation. Somatic point mutations in BRAF occur in approximately 8% of human cancers, most frequently in melanoma, thyroid and colon carcinomas [26].

RAS/RAF/MEK/ERK pathway in NENs

In NENs, both BRAF and KRAS mutations were reported to be rare or absent [27–30]. Additionally, it has been shown that human NENs, including small cell lung cancer (SCLC), medullary thyroid carcinoma (MTC) and carcinoid, are lacking phosphorylated ERK, which is considered a marker of RAF-1 pathway activation. Furthermore, some literature data suggest that RAF-1 activation may inhibit growth of NENs [31]. In particular, it has been shown that the activation of the RAF-1/MEK/ERK1/2 pathway suppresses levels of Chromogranin-A and serotonin, and in a model of MTC, the ability of RAF-1 pathway activation to suppress *in vivo* tumor growth was confirmed [32,33]. Some authors have reported that RAF-1 signaling cascade activation is associated with cellular morphology and neuroendocrine phenotype in BON-1 cells [34]; however, the mechanism is unknown. One of the hypotheses includes the focal adhesion kinase (FAK), that is a cytoplasmic tyrosine kinase that binds to the sites of integrin engagement with the extracellular matrix. Focal adhesion kinase is a downstream effector of the Raf-1-MEK1/2-ERK1/2 signaling cascade and it negatively regulated the neuroendocrine and metastatic phenotype in BON cells. In other words, RAF-1 activation reduced cellular adhesion and migration of these cells with depletion of FAK protein [35].