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Review

Steering tumor progression through the transcriptional response to growth factors and stroma

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ABSTRACT

Tumor progression can be understood as a collaborative effort of mutations and growth factors, which propels cell proliferation and matrix invasion, and also enables evasion of drug-induced apoptosis. Concentrating on EGFR, we discuss downstream signaling and the initiation of transcriptional events in response to growth factors. Specifically, we portray a wave-like program, which initiates by rapid disappearance of two-dozen microRNAs, followed by an abrupt rise of immediate early genes (IEGs), relatively short transcripts encoding transcriptional regulators. Concurrent with the fall of IEGs, some 30–60 min after stimulation, a larger group, the delayed early genes, is up-regulated and its own fall overlaps the rise of the final wave of late response genes. This late wave persists and determines long-term phenotype acquisition, such as invasiveness. Key regulatory steps in the orderly response to growth factors provide a trove of potential oncogenes and tumor suppressors.

1. Introduction

Beyond deep understanding of mechanisms underlying tumor initiation and progression, contemporary cancer research ultimately strives to develop more efficacious and selective anti-tumor drugs. In the last decade, this avenue of intense research has been inspired by the 'oncogene addiction' theory [1]. Accordingly, cancers that contain multiple genetic and chromosomal abnormalities are dependent on or 'addicted' to one or a few genes for maintenance of the malignant phenotype. Thus, reversal of only one or a few of these abnormalities can inhibit cancer cell growth and translate to improved survival rates. An example is provided by the multiple mutant forms of the epidermal growth factor receptor (EGFR) in lung tumors. Low molecular weight inhibitors of the EGFR's kinase effectively inhibit lung tumors when they express one of the mutant, constitutively active forms of EGFR [2]. Another 'addiction' might be exemplified by breast cancers that overexpress HER2, a kin of EGFR, which are effectively controlled by a monoclonal anti-HER2 antibody [3]. While cancer genome sequencing initiatives continue to identify more mutant forms and candidates for targeted therapies, the remarkable multiplicity of mutations in solid tumors [4], along with inherent adaptive mechanisms that lead to patient resistance [5], set formidable limits to the 'oncogene addiction' strategy.

It is worthwhile noting that several, rather effective cancer drugs target non-mutated cellular components. They include the estrogen receptor in breast cancer, the microtubule network in a variety of tumors, and the vascular endothelial growth factor (VEGF) in colorectal and other cancers. Moreover, anti-EGFR antibodies effectively inhibit colorectal tumors despite the fact that EGFR in these malignancies is not usually amplified or mutated. Interestingly, the presence of amphiregulin and epiregulin, two ligands of EGFR, in colorectal tumors predicts response to anti-EGFR antibodies [6]. This suggests that the therapeutic antibody achieves impact by blocking autocrine or paracrine, stroma-mediated loops involving EGFR and one of its seven ligands. Consistent with this interpretation, growth factors play essential roles in most phases of tumor progression, including clonal fixation of oncogenic mutations, recruitment of blood and lymph vessels to the growing tumor and enhancing dissemination of tumor cells, leading to colonization of distant organs (metastasis) [7]. For example, an in vivo genetic screen for genes that enhance metastasis of breast cancer to lungs identified two ligands of EGFR [8].

Another, very important contribution of growth factors to tumor progression entails chemotherapy- and radiotherapy-induced autocrine loops that permit drug resistance. For example, by generating cisplatin-resistant MCF-7 cells, it was found that drug resistance associates with increased EGFR phosphorylation, high levels of AKT1 activity, inactivation of the p53 pathway and up-regulation of amphiregulin expression [9]. Moreover, knockdown of amphiregulin expression by specific short interfering

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RNA resulted in a nearly complete reversion of the resistant phenotype. In conclusion, in-depth understanding of the roles played by the stroma and growth factors in tumor progression might identify novel candidates for therapy and for combination treatments aimed at delaying the onset of resistance to drugs. Driven by this motivation, our mini-review highlights cytoplasmic and nuclear actions of growth factors, with an emphasis on transcriptional regulation by EGFR, a relatively well-understood growth factor receptor system.

2. Cytoplasmic signaling pathways activated by growth factors (see Fig. 1)

Growth factors bind to and activate receptors at the plasma membrane [31]. In the case of EGFR, receptor activation upon ligand binding involves formation of homo- and hetero- dimers between EGFR and the other members of the ERBB family. Following ligand-induced dimerization, EGFR family members phosphorylate each other to induce full activation of their kinase domains. Other receptor tyrosine kinases (RTKs) have slightly different mechanisms of activation, but the end result is the activation of their intracellular tyrosine kinase domain. The main substrate of the EGFR kinase is itself; it phosphorylates a series of tyrosines in a long and probably unstructured carboxyl-terminal tail extending past the kinase domain. Other RTKs, however, such as the insulin and the insulin-like growth factor 1 (IGF1) receptors recruit and extensively phosphorylate adapter molecules such as IRS, GAB and FRS. In either case the phosphotyrosines serve as a platform for the recruitment of downstream signaling complexes [11].

Major pathways downstream of EGFR and RTK activation in general include the mitogen-activated protein kinase (MAPK) and the phosphatidylinositol 3-kinase-protein kinase B (PI3K-AKT) routes [12]. EGFR carboxyl-terminal phosphotyrosines recruit upstream components of the MAPK/ERK pathway, such as Grb2 and Shc [13]. These adaptors bind to the phosphorylated EGFR and recruit SOS. SOS exchanges GDP for GTP in Ras to activate Ras, and active Ras then binds to and allosterically activates the Raf kinase. Raf is the first member of a cascade of three kinases in the MAPK pathway, with Raf activating MEK and MEK activating the terminal MAPK, Erk [14]. RTK activation of the PI3K pathway begins by recruitment of a class I PI3K to phosphotyrosines generated by the activated receptor [15]. PI3K phosphorylates the relatively abundant phosphoinositide, phosphatidylinositol 4,5-bisphosphate (also known as PI(4,5)P_2 or simply PIP_2) to generate PI(3,4,5)P_3 (also referred to as PIP_3). PIP_3 recruits the Akt kinase to the membrane, where it is activated by phosphorylation by PDK1 and the mTOR complex 2, mTORC2 [16]. Akt itself phosphorylates several substrates that inhibit apoptosis, including BAD, the p53 regulator MDM2 and members of the FoxO family of transcription factors. The other major role of Akt is to activate mTORC1 through a series of biochemical steps beginning with the phosphorylation of TSC2 in the TSC1/TSC2 complex. Phosphorylation

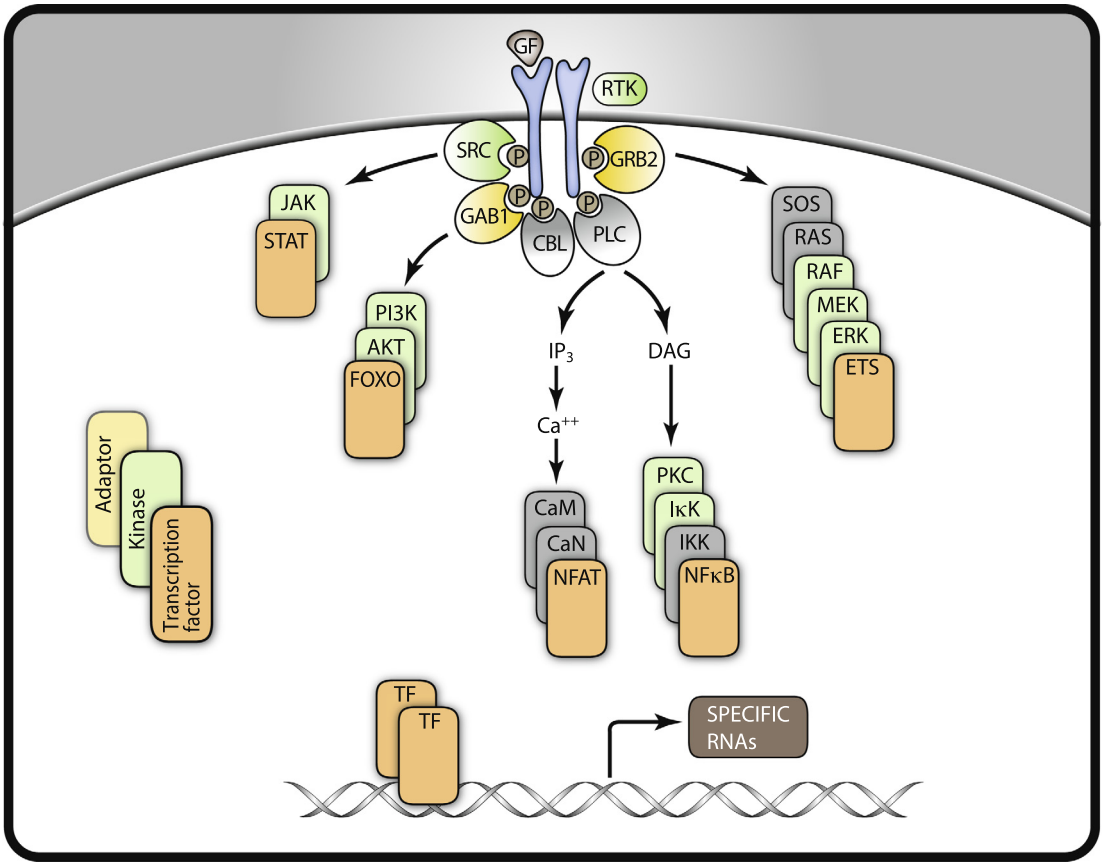

Fig. 1. Receptor-mediated signaling and the initiation of transcriptional regulation. Receptor tyrosine kinases (RTKs), activated by growth factors (GFs) signal through several pathways to drive changes in the transcription of specific RNAs. Adapters (yellow), kinases (green) and other signaling proteins (gray) bind to phosphorylated tyrosines (encircled P letters) on the activated RTK, and then stimulate particular downstream pathways. For instance, upon binding to phosphotyrosine, phospholipase C, PLC, is activated to cleave the membrane lipid phosphatidylinositol 4,5-bisphosphate, PIP_2, into inositol 1,4,5-trisphosphate, IP_3, and diacylglycerol, DAG. Pathway activation culminates in changes in transcription factor (TF) activity, leading to changes in RNA expression. The extent of activation of each of these pathways will vary depending on the identity of the RTK. Also shown is the negative regulator CBL, which binds to phosphorylated tyrosines and ubiquitinates the RTK driving its internalization. Other RTK specific negative regulators such as MIG6 are not shown.

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