ability of receptor tyrosine kinase signaling to augment cell survival as well as proliferation. Inhibitors of EGFR and ALK produce similar therapeutic responses in patients with lung adenocarcinomas harboring *ERBB1* mutations or *EML4-ALK* fusion genes, respectively.

Unfortunately, none of these targeted therapies cure advanced lung cancer. In treated patients tumors have often been found to have acquired activating mutations in some other signaling molecule, most often another tyrosine kinase, which sidesteps the effects of the drug, resulting in resistance to the targeted therapy. For example, lung cancers that develop resistance to EGFR inhibitors often have amplifications in a gene called *MET*, which encodes yet another receptor tyrosine kinase. This experience highlights one of the most daunting clinical problems in the treatment of advanced cancers—the presence of subclones within the genetically heterogeneous tumor cell population that are resistant to targeted therapies.

Downstream components of the receptor tyrosine kinase signaling pathway. As mentioned, receptor tyrosine kinase activation stimulates RAS and two major downstream signaling "arms," the MAPK cascade and the PI3K/AKT pathway. In line with the importance of these pathways in mediating cell growth, RAS, PI3K, and other components of these pathways are frequently involved by gain-of-function mutations in different types of cancer. Of interest, when *RAS* mutations are present in a tumor, activating mutations in receptor tyrosine kinases are almost always absent, at least within the dominant tumor clone, implying that in such tumors activated RAS can completely substitute for tyrosine kinase activity. Thus, lung adenocarcinomas fall into mutually exclusive molecular subtypes that are associated with mutations involving *RAS* or various tyrosine kinase genes, an insight that has important implications for targeted therapies in this type of cancer.

RAS Mutations. **Point mutations of *RAS* family genes constitute the most common type of abnormality involving proto-oncogenes in human tumors**. The *RAS* genes, of which there are three in humans (*HRAS*, *KRAS*, *NRAS*), were discovered initially in transforming retroviruses. Approximately 15% to 20% of all human tumors express mutated RAS proteins, but in some types of cancers the frequency of *RAS* mutations is much higher. For example, 90% of pancreatic adenocarcinomas and cholangiocarcinomas contain a *RAS* point mutation, as do about 50% of colon, endometrial, and thyroid cancers and about 30% of lung adenocarcinomas and myeloid leukemias. *RAS* mutations are not nearly as prevalent in other kinds of cancers, but are nevertheless detected at low frequencies in most.

Recall that RAS proteins are members of a family of membrane-associated small G proteins that bind guanosine nucleotides (guanosine triphosphate [GTP] and guanosine diphosphate [GDP]), similar to the larger trimolecular G proteins. They normally flip back and forth between an excited signal-transmitting state in which they are bound to GTP and a quiescent state in which they are bound to GDP. Stimulation of receptor tyrosine kinases by growth factors leads to exchange of GDP for GTP and subsequent conformational changes that generate active RAS, which in turn stimulates both the MAPK and PI3K/AKT arms of the receptor tyrosine kinase signaling pathway. These downstream kinases phosphorylate and activate a number of cytoplasmic effectors as well as several transcription factors that turn on genes that support rapid cell growth. Activation of RAS is transient because RAS has an intrinsic GTPase activity that is accelerated by *GTPase-activating proteins (GAPs)*, which bind to the active RAS and augment its GTPase activity by more than 1000-fold, thereby terminating signal transduction. Thus, GAPs prevent uncontrolled RAS activity.

Several distinct RAS point mutations have been identified in cancer cells that markedly reduce the GTPase activity of the RAS protein. As a result, these mutated forms of RAS are trapped in the activated GTP-bound form and the cell receives pro-growth signals continuously. It follows from this scenario that the consequences of gain-of-function mutations in RAS proteins should be mimicked by loss-of-function mutations in GAPs that normally restrain RAS activity. Indeed, disabling mutations of neurofibromin 1, a GAP encoded by the *NF1* gene, are associated with the inherited cancer syndrome *familial neurofibromatosis type 1* (Chapter 25). *NF1* is therefore an example of a tumor suppressor gene that acts through negative regulation of RAS signaling.

Oncogenic BRAF and PI3K Mutations. In addition to RAS, other downstream factors in the receptor tyrosine kinase signaling pathway are frequently involved by gain-of-function mutations in various cancers.

- **Mutations in *BRAF***, a member of the *RAF* family, have been detected in close to 100% of hairy cell leukemias, more than 60% of melanomas, 80% of benign nevi, and a smaller percentage of a wide variety of other neoplasms, including colon carcinomas and dendritic cell tumors. BRAF is a serine/threonine protein kinase that sits at the top of a cascade of other serine/threonine kinases of the MAPK family. Like activating RAS mutations, activating mutations in BRAF stimulate each of these downstream kinases and ultimately activate transcription factors. Mutations in other MAPK family members downstream of BRAF are uncommon in cancer, suggesting only mutations affecting factors near the top of the RAS/MAPK cascade produce significant pro-growth signals in most cell types.
- **Mutations of the PI3K family of proteins** are also very common in certain cancers. PI3K is heterodimer comprised of a regulatory subunit and a catalytic subunit, of which several tissue-specific isoforms exist. Under normal circumstances, PI3K is recruited by receptor tyrosine kinase activation to plasma membrane-associated signaling protein complexes. Here, like BRAF, it activates a cascade of serine/threonine kinases, including AKT, which is a key signaling node. AKT has many substrates, several of which are particularly important. mTOR, a sensor of cellular nutrient status, is activated by AKT and in turn stimulates protein and lipid synthesis. BAD is a pro-apoptotic protein that inactivated by AKT, an effect that enhances cell survival. Similarly, FOXO transcription factors, which turn on genes that promote apoptosis, are also negatively regulated by AKT phosphorylation. Like RAS, PI3K is negatively regulated by an important "braking" factor called *PTEN*. Alterations in virtually all components of the