

Emerging Role of RAB GTPases in Cancer and Human Disease

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Abstract

Emerging evidence implicates alterations in the RAB small GTPases and their associated regulatory proteins and effectors in multiple human diseases including cancer. We have recently shown that *RAB25*, located at chromosome 1q22, is amplified at the DNA level and overexpressed at the RNA level in ovarian and breast cancer. These changes correlated with a worsened outcome in both diseases. In addition, enforced expression of *RAB25* in both breast and ovarian cancer cells decreased apoptosis and increased proliferation and aggressiveness *in vivo*, potentially explaining the worsened prognosis. A better understanding of genetic alterations as well as the physiologic and pathophysiologic roles of RAB GTPases may open new opportunities for therapeutic intervention and better outcomes. (Cancer Res 2005; 65(7): 2516-9)

Molecular Profiling and Identification of Genetic Defect in Cancers

Cancer is caused by abnormalities in DNA sequence, copy number, rearrangements, or expression. The accumulation of multiple changes in critical genes within a single cell is required to escape from normal controls on cell growth and proliferation, allowing development into a clinically evident tumor (1). Understanding the molecular basis of tumorigenesis is the key to better diagnosis, prognosis, and treatment for cancer. Large-scale profiling of gene expression and genomic alterations has revealed multiple differences between normal and malignant cells, specific genetic and cellular changes that occur at each stage of tumor progression, and aberrations that can distinguish cancers of different origins and metastatic potential. This genome-wide information will facilitate the identification of new diagnostic markers, prognostic and predictive information, and novel molecular targets. This, in turn, will lead to the development, validation, and implementation of new, less toxic, and more effective drugs.

Array comparative genomic hybridization (2) provides a robust, sensitive, and high-resolution approach to the identification of regions of DNA copy number increase and decrease in tumors. These copy number aberrations are selected during tumorigenesis, contributing to the behavior of tumors, and indeed can be predictive of patient outcome (3). Multiple chromosomal amplifications implicated in the pathophysiology of ovarian and breast carcinomas have been detected (4, 5). The identification of the candidate genes driving the development of the DNA copy number aberrations in cancer has progressed at a slow rate. However, new technologies are likely to increase the pace. We, for example, have

extensively characterized an amplicon present in ovarian cancer on chromosome 3q, finding that the *PIK3CA* gene expressing the catalytic α subunit of phosphoinositide-3-kinase is amplified in ovarian cancer and is a critical regulator of different functions in ovarian cancer (6). This, along with other developments, has contributed to the phosphoinositide-3-kinase pathway being identified as a prime candidate for the development of novel therapies.

RAB25 Overexpression Drives Ovarian Cancer Progression

Array comparative genomic hybridization revealed a frequent increase in a 1.1 mb region at chromosome 1q22 in 54% and 47% of advanced serous epithelial ovarian cancers and breast cancers, respectively (7), suggesting the potential involvement of a gene within this region in breast and ovarian cancers. A total of 34 open reading frames, including 22 known genes and 12 hypothetical proteins, reside in this region. Using the powerful resource of publicly available gene expression data sets, generously made available by colleagues (8), we were able to eliminate 18 genes as candidates as they did not show frequent changes in gene expression. The remaining 16 candidates were studied by real-time quantitative PCR. Although mRNA levels of several of the genes in this region were modestly elevated in a fraction of ovarian cancers as compared with normal ovarian surface epithelial cells or benign ovarian tumors, *RAB25* mRNA levels were significantly increased in 89% ($P < 0.001$) of the ovarian cancers analyzed. In an independent ovarian cancer data set (9), *RAB25* levels were increased in 80% of ovarian cancer samples compared with normal ovarian epithelium. Similarly, *RAB25* was overexpressed in 67% of the tumors from breast cancer patients when compared with normal breast tissue (10). Linear regression analysis showed a direct relationship between DNA copy number and RNA expression; however, the increase in RNA levels occurs with a greater frequency than the increase in DNA copy number, suggesting that additional mechanisms could lead to deregulation of *RAB25* mRNA levels.

Ovarian cancer patients with elevated 1q22 either failed to enter a disease-free state following surgery and chemotherapy or exhibited short durations of disease-free survival, suggesting that a gene in this region regulates the behavior of ovarian cancer. Indeed, ovarian cancer patients with high *RAB25* mRNA levels (>2-fold increase compared with normal controls) showed a decrease in disease-free interval and overall survival. Similarly, Kaplan-Meier survival analysis of the 109 patients with breast cancer, for whom follow-up data was available in the data set at the Stanford web site (http://genome-www.stanford.edu/breast_cancer/robustness/data.shtml), revealed a correlation between high *RAB25* expression and a decrease in both overall survival ($P < 0.02$) and disease-free survival ($P < 0.01$). Together, these data suggest that *RAB25* contributes to the aggressiveness of breast and ovarian cancers. Mutations in *RAB25* were not detected in ovarian cancer;

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however, whether *RAB25* is mutated in other tumors remains to be determined. Indeed, the *PIK3CA* gene, which is amplified in serous epithelial ovarian cancer (6), is frequently mutated in endometrioid ovarian cancers, brain, lung, and breast tumors (11, 12).

The role of *RAB25* in the aggressiveness of ovarian and breast cancers was assessed by altering *RAB25* levels in cell lines. Elevating the expression of *RAB25* in ovarian and breast cancer cell lines increased anchorage-independent colony-forming activity, cell proliferation, and cell survival under multiple stress conditions including serum starvation, anchorage deprivation, UV irradiation, and chemotherapy treatment (paclitaxel). Decreasing *RAB25* expression by small interfering RNA decreased cell proliferation and markedly increased the sensitivity to apoptosis of both breast and ovarian cancer cells. In murine xenograft assays, *RAB25* facilitated tumor formation and increased the growth rate of ovarian cancer cells. However, whereas *RAB25* overexpression was sufficient to make cancer cells more aggressive, in contrast to RAS (13), *RAB25* overexpression could not cooperate with SV40 large T antigen and hTERT to drive tumor formation by immortalized human cells. Thus, *RAB25* seems to play a role in tumor progression and aggressiveness rather than tumor initiation, a concept compatible with the increases in *RAB25* levels at later stages in ovarian cancer.

Analysis of the antiapoptotic effects of *RAB25* revealed Bcl-2 and phosphoinositide-3-kinase pathways that are known to regulate cell survival (14, 15). We found that enforced *RAB25* expression in ovarian cancer cells not only decreases levels of the BAX and BAK proteins, two key effectors of mammalian apoptotic signaling cascade (16), but that *RAB25* also increases AKT phosphorylation, an indication that the phosphoinositide-3-kinase pathway is active. Down-regulating *RAB25* reversed the decrease in BAX and BAK levels and the increase in AKT activity, supporting the potential involvement of the Bcl-2 and phosphoinositide-3-kinase pathways in mediating *RAB25* action.

Intercellular Vesicle Trafficking Pathways: A Potential Therapeutic Target?

Information arising from global DNA, RNA, and protein analysis will not only aid in unraveling the initiation, development, and progression of genetic diseases such as cancer, but will also facilitate the identification of new biomarkers and molecular targets. This will rapidly open a new chapter in drug discovery, development, and patient care. Using this approach, we are able to identify the driver of an important chromosomal amplification at 1q22 in breast and ovarian cancer. The amplification at 1q22 had been previously shown to be present in breast cancers (17) but had not been noted in ovarian cancer. However, in breast cancer, the region of genomic aberration is large, encompassing much of 1q. In ovarian cancer, the regional amplification was quite narrow and thus, missed by chromosomal comparative genomic hybridization. The narrow region of amplification, as well as the resolution of array comparative genomic hybridization, facilitated the identification of *RAB25* as a driver of the aberration at 1q22. Indeed, since the publication of our paper, we have done higher resolution array comparative genomic hybridization and have further delimited the region of aberration in ovarian cancer, demonstrating a narrow region of amplification at 1q22. The mechanisms contributing to the different structures of the 1q amplicon in breast and ovarian cancer warrants further investi-

gation to determine whether this reflects different mechanisms leading to genomic instability or the selection of additional genes in breast cancer. In addition to breast and ovarian cancers, Wilms' tumors also show an amplification at 1q22 (18); however, whether this involves *RAB25* remains to be determined. Nevertheless, increased copy number at chromosome 1q22 is associated with a high relapse rate in invasive ductal breast carcinomas (17) and Wilms' tumors (18). In addition, Kudoh et al. (19) have reported that a significantly higher ratio of genetic changes, characterized by increased copy number of chromosome 1q21-q22 and 13q12-q14, are present in cisplatin-resistant than in cisplatin-sensitive ovarian tumors. Together, these data suggest that a gene(s) located at 1q22 controls tumor progression, aggressiveness, and potentially, chemosensitivity. Our studies revealed *RAB25* as a major player in the development of the 1q22 amplicon and in tumor behavior.

RAB GTPases play a master role in regulating intercellular vesicle trafficking in both exo- and endocytic pathways. Approximately 60 human RAB genes are encoded by the human genome. However, the complexity of the RAB protein family might be even greater as there is evidence that alternative splicing of RAB mRNAs results in the production of functionally distinct isoforms (20). Most RAB proteins are constitutively expressed in all mammalian cells, although several RAB proteins have been shown to be differentially expressed in epithelia and neurons, perhaps fulfilling specialized transport functions of these polarized cells. RAB protein function is regulated at multiple levels including protein expression, localization, activation, binding partners, and recruitment of specific downstream effectors. Recent studies have shown multiple links between RAB GTPase dysfunction and associated regulatory proteins in human diseases. For instance, Griscelli syndrome type 2, caused by mutation of the *RAB27a* gene (21), is a rare autosomal recessive disorder that results in pigmentary dilution of the skin and hair, the presence of large clumps of pigment in hair shafts, and an accumulation of melanosomes in melanocytes. Most Griscelli syndrome type 2 patients also develop an uncontrolled T lymphocyte and macrophage activation syndrome, known as hemophagocytic syndrome, leading to death in the absence of bone marrow transplantation. Altered function of multiple RAB regulatory proteins, such as RAB escort protein, RAB geranylgeranyl transferase, and RAB GDP dissociated inhibitor, causes choroideremia (retinal degeneration), Hermansky-Pudlak syndrome (a type of albinism which includes a bleeding tendency and lung disease), and X-linked nonspecific mental retardation, respectively (20).

Dysregulation of RAB gene expression may be a generalized component of human tumors. In addition to *RAB25*, many RAB proteins exhibit elevated expression at the RNA level. RNA microarray analyses in ovarian cancer show that ~45% to 50% of known RAB or RAB-associated genes display increases in mRNA expression. Moreover, these increases are frequent, occurring in over two-thirds of tumor samples. Up-regulation of *RAB5a* and *RAB7* occurs in thyroid-associated adenomas (22). We have shown an increase in *RAB25* gene expression in ovarian and breast cancers. Increased *RAB25* levels have also been noted in prostate cancer (23), transitional cell carcinoma of the bladder (24), and invasive breast tumor cells (25), suggesting the pathologic role of RAB proteins in the development or progression of tumors in multiple epithelial lineages.

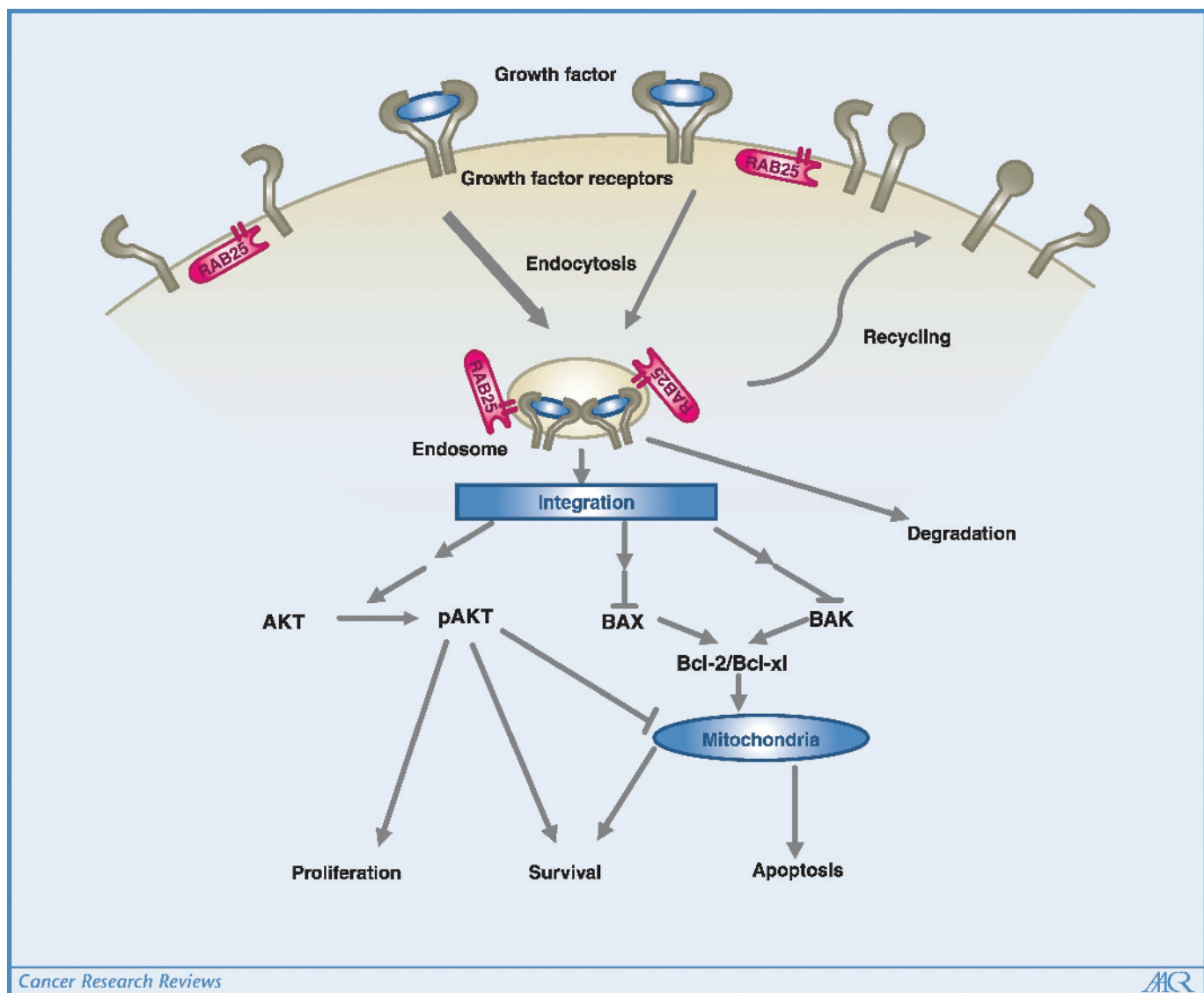


Figure 1. Proposed mechanism of RAB25 regulation of cell proliferation and survival. In resting cells, RAB25 and growth factor receptors are located on the cell surface. Activation of growth factor receptors by their cognate ligands results in receptor internalization. RAB25 proteins regulate receptor internalization, formation of vesicles, trafficking to specific cellular compartments including the nucleus, the proteasome, lysosome, and recycling to the cell surface. The intracellular vesicles may also provide nodes for the interaction of multiple signaling molecules allowing signal integration. The most likely role for RAB25 is in determining the efficiency of endosome recycling and in targeting the endosome to a particular intracellular compartment. RAB25 expression results in activation of AKT and down-regulation of BAX and BAK levels through as yet unknown mechanisms. This, in turn, results in increased proliferation, decreased apoptosis, and increased aggressiveness *in vivo*.

It is likely that additional evidence will accumulate implicating RAB dysfunction in cancer and other diseases, as RAB proteins have crucial roles in vesicle trafficking, signal transduction, and receptor recycling which in turn regulate normal cellular activity (Fig. 1). For example, a recent study has provided direct evidence that multiple signaling molecules including AKT, extracellular signal-regulated kinase 1/2, and p38 mitogen-activated protein kinase associate on endocytic vesicles and mediate their function by facilitating translocation of the vesicles to the nucleus, implicating RAB GTPases in conducting cell survival signals (26). In addition, RAB7 has been shown to mediate endocytosis and degradation of glucose and amino acid transporters in growth factor-deprived conditions, which in turn control cellular nutrient uptake and sensitivity to cell death (27). Based on the prevalence of altered *RAB* expression and function in many diseases, it is of interest to consider the therapeutic potential of

controlling RAB function or RAB-regulated pathways through pharmacologic or genetic modulation.

In conclusion, the RAB GTPase family may be involved in a variety of disease settings including cancer progression. As the mechanisms of their actions are identified, RAB GTPases and the signaling and trafficking pathways in which they operate may present novel targets for therapeutic invention.

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