Rho GTPases as early markers for tumour progression

Tumour-cell invasion and metastasis require a complex cascade of events that includes loss of cell-to-cell contacts, increased cell migration, degradation of extracellular matrix, and increased cell survival and proliferation. Cells that have been transformed by gaining expression or function of molecules mediating these events have a clear advantage in tumour progression and metastasis. A study by Manoj Abraham and colleagues\(^1\) is one of the first to show that members of the Rho family of small GTPases, and some of their downstream effectors, may be potential diagnostic indicators for malignancy.\(^2\) Abraham and colleagues found that Rho A and a related molecule, Rac 2, are overexpressed in head and neck squamous-cell cancers, as well as in malignant squamous-cell cancer cell-lines.\(^3\) They found a similar pattern of expression for another Rho family member, Cdc42, as well as for a downstream effector, phosphatidyl inositol 3-kinase (PI3K), and the actin-nucleating molecules, Arp 2 and 2E4/Kaptin.\(^4\) Even moderately differentiated tumours had raised Rho and Rac concentrations, which suggests that these molecules may be early markers of tumour progression. Only a handful of studies\(^5,6\) have examined the expression of Rho-like molecules in tumour samples.

Rho, Rac, and Cdc42 are, like the other members of the Ras superfamily, to which the Rho family of small GTPases belong, acting as molecular switches that alternate between a GDP-bound inactive state and a GTP-bound active state (figure). Activation of Rho and Ras proteins is regulated downstream of growth-factor receptors and adhesion molecules via guanine nucleotide exchange factors (GEFs), which catalyse the exchange of GDP for GTP. Binding to GTP causes a conformational change in the GTPase that allows it to bind and activate several downstream effectors and initiate several signalling pathways. The cascade is turned off by the action of GTPase-activating proteins (GAPs), which catalyse the intrinsic GTPase activity of the Rho and Ras family members. Several of the GEFs that activate Rho family members, such as Tiam-1, Dbl, Ost, and Vav, are known oncogenes.\(^7\)

A key function of members of the Rho family is regulation of the actin cytoskeleton. Rho regulates the formation of actin stress fibres and the generation of actin-myosin-based contractile forces in cells. Rac and Cdc42 have been implicated in actin dynamics found at the leading edge of migratory cells and associated with the formation of filopodia and lamellipodia. Cdc42 works in part through its effector, N-WASP (N isoform of Wiskott-Aldrich syndrome protein), which binds to the Arp 2/3 complex, allowing it to nucleate actin filaments.\(^8\) Arp 2/3 has also been implicated in the branching of actin filaments near lamellipodia,\(^9\) regulation of which affects the dynamics and structure of the actin cytoskeleton. Rac and Cdc42 activate p21-associated kinases (PAKs), which are also important in the formation of lamellipodia.\(^9\) They also activate P13K, a lipid kinase that phosphorylates the D3 position of the inositol ring, to create novel lipid second messengers, PI(3,4)P\(_2\) and PI(3,4,5)P\(_3\), PI3K has
been implicated in many signalling pathways, including regulation of the actin cytoskeleton, activation of GEFs for Rho family members, activation of Akt (which leads to cell survival), and regulation of cell migration.18 Overexpression of Rho family members increases migration and invasiveness in various cell lines and culture models,19,20 which suggests that such overexpression in carcinomas could profoundly affect metastatic potential in vivo. In addition, an in-vivo screen for metastasis-associated genes suggests a role for RhoC in this process.12 The exact molecular basis for the effects of the Rho family on cell migration is not entirely known, but clearly relates to regulation of the actin cytoskeleton. The function of PI3K seems to be crucial.13,21 Moreover, it is increasingly apparent that Rho GTPases participate in and regulate signalling pathways downstream of the integrin family of cell-adhesion receptors, which are the very molecules that mediate cellular attachment to and migration across connective-tissue components of the extracellular matrix. Tumour-cell invasion requires not only cellular migration, but also often involves the activation of proteases that remodel the extracellular matrix. Recent evidence suggests that members of the Rho family can regulate matrix metalloproteinases (MMPs) as well. For example, activation of Rac can activate MMP-2 (collagenase IV)22 and can upregulate expression of collagenase-1.15 Therefore, overexpression of Rho GTPases is likely to increase tumour invasiveness through effects on both cell migration and matrix degradation. Since many of the molecules that promote cell migration are also positive regulators of cell proliferation or survival, they would provide invasive tumour cells with a growth advantage when these cells metastasise. Rho family members promote cell-cycle progression by regulating cyclin D1, p21,23,24, p27,25, and transcription factors such as NFκB.16 Cell survival is also promoted by Rho GTPases, in part through the activation of PI3K and subsequent activation of Akt/PKB, which prevents apoptosis.17 This pathway suggests that enhanced expression of Rho GTPases or PI3K could correlate with tumour progression and poor clinical outcome in part through effects on cell growth. Before cancer cells can start to invade surrounding tissue, their connections to neighbouring cells must be lost. Cell-cell contacts not only regulate cellular differentiation, but also suppress migration and proliferation. It is therefore significant that Rho, Rac, and Cdc42 regulate the formation of cell-cell contacts and adherens junctions.18 Overexpression of Rho GTPases has the potential to dysregulate these contacts, and provide a further mechanism by which Rho, Rac, or Cdc42 might enhance cell migration, invasion, and proliferation. Because cell migration, invasion, cellular proliferation, and survival are advantageous to tumour cells for the formation of metastases, the finding that Rho family GTPases and PI3K are overexpressed in squamous-cell cancers is likely to be relevant to tumour progression. Early tumour markers associated with invasiveness or metastasis have important potential as a diagnostic tools, and they may also be useful for the development of prognostic indicators and novel therapeutic strategies.

**Is breast MRI mature enough to be recommended for general use?**

Magnetic-resonance imaging (MRI) has transformed the investigation of diseases of the neurological and musculoskeletal systems, since other techniques were poor at imaging neural tissue, muscles, and tendons. The use of MRI in oncology has followed more slowly for several reasons: MR images of tissues with cancer may not provide any further information than do other techniques, such as computed tomography, ultrasonography, and mammography; as with other radiological technique, the identification of cancer from an image is unreliable; and probably because of these factors, access for oncology patients is limited by demands by neurology and orthopaedics.

Rapidly developing radiological technology means that there is limited time to evaluate or carry out randomised controlled trials of a technique. The UK Royal College of Radiologists has published evidence-based guidelines1 on the use of radiological procedures. In the 4th edition, only five of 15 indications about imaging of breast disease came from randomised controlled trials (category A). Mammography has a research base unrivalled in radiology, since its effectiveness is shown by randomised controlled trials based on whole populations and with up to 30 years’ surveillance.2 The age at which patients should be screened, number of views, reports, and frequency of screening by conventional mammography have all been investigated in randomised studies. Such

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17 Vanhaesebroeck B, Alessi DR. The PI3K-PDK1 connection: more than just a road to PKB. *Biochem J* 2000; 346: 561–76.