

# Pathogenesis of endocrine thyroid cancer

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## Abstract

This review aims to discuss the different genetic alterations that may come about and thus give rise to thyroid cancer. The importance of understanding the pathogenesis of this disease is in the use of these genetic alterations as prognostic markers and as targets in treatment. Principal alterations to these central pathways, namely the MAPK pathway and PI3K/AKT pathway, are mutations, increase in gene number, methylations and translocations. The effects of the environment on the progression of thyroid cancer, such as the effects of the microenvironment and exposure to endocrine disruptors, will also be discussed.

## Keywords

Thyroid cancer, pathogenesis, signalling pathways

## Introduction

While the prevalence of most cancers has remained the same or decreased, the incidence of thyroid cancer has increased.<sup>1</sup> Therefore there is a great need for us to understand the mechanism of this disease since this may help us understand and determine the factors that are causing this increase in incidence. More importantly we hope that by understanding the pathogenesis of thyroid cancer we will be one step closer in finding a cure or at least a better treatment for this disease. The majority of thyroid cancers are derived from follicular cells, namely papillary carcinomas (PTC), follicular carcinomas (FTC), anaplastic carcinomas (ATC) and poorly differentiated thyroid cancers (PDTC). The remaining of thyroid cancers arise from parafollicular cells (also known as C cells) and are known as medullary thyroid cancers (MTC).<sup>2</sup>

## Mutations

A mutation is a permanent change in the DNA sequence of a gene, this may be somatic or germline. A somatic mutation is one that occurs in somatic tissue i.e. any of the cells body except germ cell and so cannot be passed on to future generations. Germline mutations occur in germ cells and so can be passed on to future generations. Two of the most common genetic mutations in thyroid cancer are those of *BRAF* and *RAS*, both of which are somatic mutations. *BRAF* is a gene coding for a proto-oncogene, the mutant form being BRAF-V600E. This form of mutation tends to be common and is commonly associated with papillary thyroid cancer. *BRAF* mutations have been shown to be related with poor clinicopathological outcomes and have a high risk of recurrence. It is also associated with metastasis of the lymph nodes, advanced stages of cancer and extrathyroidal extensions. BRAF-V600E leads to the continuous activation of the serine/threonine kinase, also known as the MAP kinase pathway (MAPK). *BRAF* mutations are associated with an aggressive form of PTC, this is not seen in other mutations such as that of *RAS* (both of which are involved in the constitutive activation of MAPK pathway). This may come about as a result of the increased production of tumour promoting molecules such as vascular endothelial growth factor (VEGF), matrix metalloprotein, proinhibitin, Ki-67 and c-MET. BRAF-V600E is also associated with the methylation of the promoters of several tumour suppressor genes and therefore the decreased expression of the tumour suppressor genes. Both of these events make PTC particularly aggressive.<sup>3</sup>

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*RAS* mutation is another very common mutation in thyroid cancer, following *BRAF*, but tends to be less aggressive.<sup>2</sup> *RAS* proteins are GTPase proto-oncogenes, that are important in the regulation of the MAPK and PI3K/AKT pathways.<sup>4</sup> The *RAS* mutation is associated with the transformation of follicular thyroid adenoma, which is a premalignant lesion, in thyroid cancer.<sup>2</sup> PTCs with the *RAS* mutation tend to be encapsulated and have a lower rate of lymph node metastasis.<sup>4</sup>

### Methylation

Methylation is the addition of a methyl group to DNA. Hypomethylated genes are found to be over expressed in the presence of the BRAFV600E mutation. The hypo- or hyper-methylation of genes arises from signals that come about from the MAPK pathway driven by a mutation.<sup>5</sup> The hypermethylation of promoters seems to be commonly associated with thyroid cancers and is the most common mechanism for the inactivation of genes. In fact methylation is also seen in the promoter of the *PTEN* gene, and is commonly seen in sporadic tumours. Normally *PTEN* acts as a tumour suppressor gene by down-regulating the PI3K/AKT pathway. The driving force of thyroid cancer from low grade to high grade is associated with the deviation of the PI3K/AKT pathway from its normal course. Thus a normally functioning *PTEN* gene prevents apoptosis. *PTEN* methylation results in the amplification and mutation of PIK3CA and is also associated with *RAS* mutations. This is commonly seen in FTC.<sup>6</sup>

### Translocations

In translocation a segment from one chromosome breaks off and reattaches to a nonhomologous chromosome or to a new site on the same chromosome. Chromosomal rearrangements involved in PTC are those of the proto-oncogenes *RET* and Neurotrophic tyrosine kinase (*NTRK1*). DNA is susceptible to strand breaks when exposed to ionising radiation, something that is commonly seen in PTC. In fact there is a relationship between radiations, RET/PTC rearrangements leading to PTC formation.<sup>7</sup> Chromosomal rearrangements come about not only because the gene loci involved have been damaged by radiation but also because they come in close vicinity during interphase of a thyroid cell.

*RET* and *NTRK1* rearrangements come about because of paracentric inversions. These events happen on chromosome 10 in the case of *RET* and on chromosome 1 in the case of *NTRK1*.<sup>8</sup> Both of these proto-oncogenes code for tyrosine kinase receptors that are found on cell membranes. *RET* acts as a receptor for the growth factor of glial cell line-derived neurotrophic factor (GDNF) family, it permits the survival of many neurones and prevents apoptosis. *NTRK1* binds nerve growth factor and is also important for the maintenance of neurons. *RET* and *NTRK1* are both normally

expressed in follicular C cells, eventually activate other cellular pathways including RAS/MAPK and PI3K. Chromosomal recombination retains the promoter and this results in the constitutive expression of the oncogene. The break point occurs between the coding sequence for the extracellular and transmembrane domain and that of the tyrosine kinase domain. Spontaneous dimerization comes about because of an intact protein-protein interaction domain. This results in ligand-independent autophosphorylation and therefore activation of downstream signalling cascades via cellular pathways. PTC can develop due to the chromosomal rearrangement of *RET*, unaided by other genetic alterations.<sup>7</sup> The relationship of radiation in thyroid cancer was studied closely in post-Chernobyl patients. It was very clear that children exposed to radiation were more likely to develop functional PTC, and were found to have RET/PTC rearrangements, amongst other genetic alterations such as mutations leading to BRAF-V600E.<sup>4</sup>

### Increases in gene number

In thyroid cancers we also have an increase in genomic number, particularly those of the RTK genes and also of genes that code for members of the PI3K-AKT pathway. These were even more evident in ATC, with the gain of the PDGFR $\alpha$ , PDGFR $\beta$ , EGFR and VEGFR1 genes. The copy gain of RTK genes may act as potential therapeutic targets. The increase of the RTK gene more commonly interacts with AKT, that is, it promotes tumourigenesis and invasiveness through the PI3K/AKT pathway in ATC.

Commonly increases in gene number are not seen as an isolated genetic alteration, but rather are seen to exist with mutations, as seen in ATC. This may aid in the irregular signalling of pathways. Genetic mutations are commonly associated with increases of the RTK genes. An exception to this is seen in FTC where PI3KCa increases in number, rather than RTK.<sup>9</sup>

IQGAP1 (Ras GTPase-activating-like protein 1) is a scaffold protein. The increase expression of its corresponding gene plays a vital role in the spread of thyroid cancer. It may also be used as a marker and therapeutic target. The IQGAP1 polypeptide is very similar to the RAS- related GTP-ase proteins. In most cancer cells it might also be able to alter the RAS  $\rightarrow$  RAF  $\rightarrow$  MEK  $\rightarrow$  MAP kinase pathway.<sup>10</sup>

### MAPK signalling pathway

An extracellular mitogen binds to a membrane bound receptor, this activates SOS which promotes the removal of GDP from RAS in exchange for GTP. This in turn activates RAF (MAP3K), which activates MAPK2, which then activates MAPK, which then activates transcription factors. Ras activates RAF which then phosphorylates and activates MEK, thus

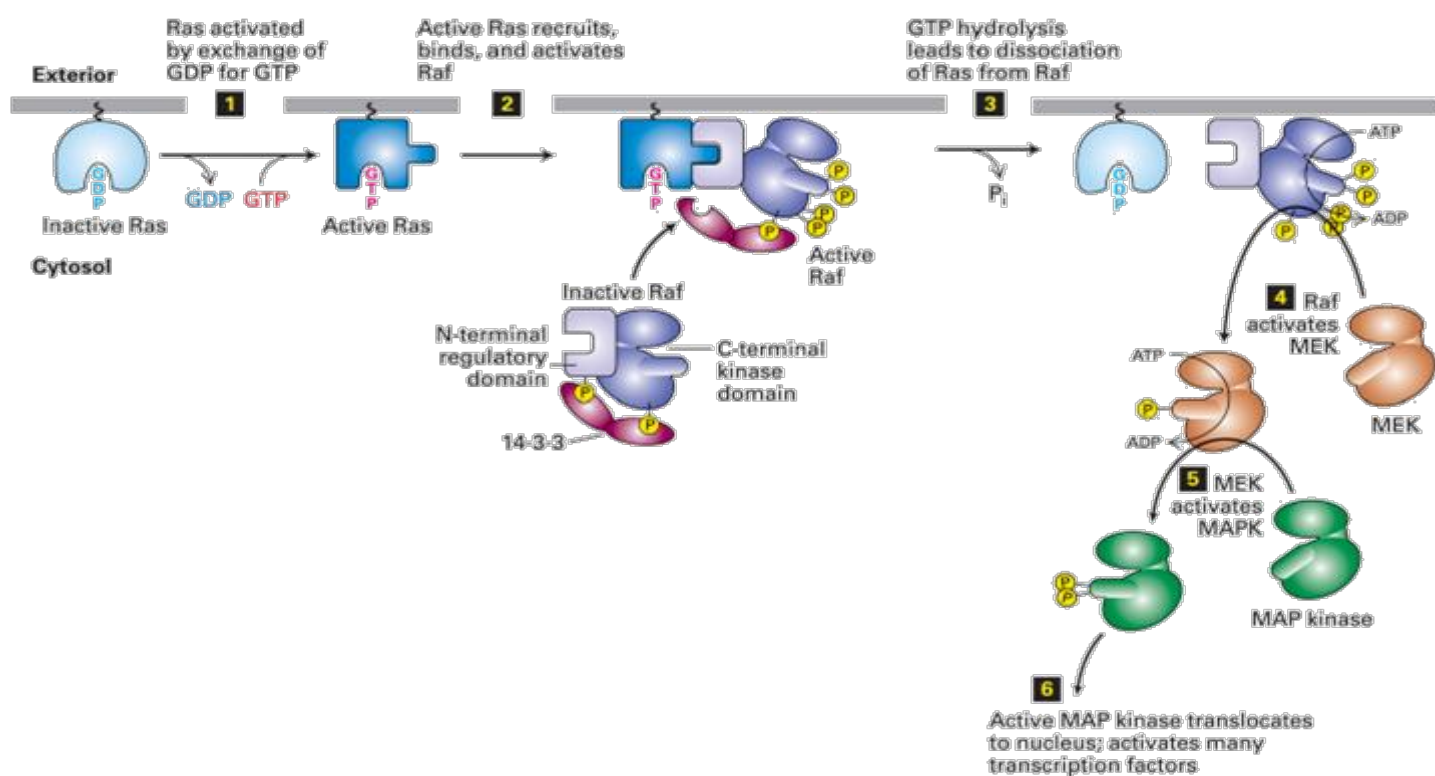
phosphorylating and activating MAPK.<sup>11</sup>

Mutant RTKs are a common genetic event in most forms of hereditary MTC. *RET* is a proto-oncogene, its activation leads to the constitutive activation of the MAPK pathway and this stimulates tumourigenesis. This hereditary mutant *RET* gene is commonly seen in patients with multiple endocrine neoplasia type 2A or 2B or familial MTC.<sup>12</sup> Constitutively active mutant RAF proteins that are typically seen in tumour cells, induce quiescent cultured cells to divide uncontrollably (even in the absence of hormones). On the other hand a constitutively active RAS protein cannot stimulate a defective RAF protein to proliferate.<sup>13</sup> The most

common mutations found in DTC are those of the *BRAF* gene. Most of the time a valine takes the place of a glutamine acid. The *BRAF* V600E mutation is associated with lymph node metastasis, extra thyroidal extension and advanced stage of the disease.<sup>12</sup> The most common genetic alteration that occurs in sporadic adult papillary carcinomas is the *BRAF* point mutation. On the other hand patients who had been previously exposed to radiation, both accidentally and for therapeutic reasons, presented with a high frequency of *RET/PTC* rearrangements, with no or little occurrence of *BRAF* point mutations.<sup>1</sup>

**Figure 1:** Diagram showing the events of the MAPK pathway. From *Molecular Cell Biology*, 7th edition, 2012

## The Ras MAP kinase pathway



### PI3K-AKT Pathway

PTEN is one of the most commonly lost tumour suppressor genes, during tumour development mutations and deletions occur that inactivate its enzyme activity to increase cell proliferation and reduce cell death. PTEN is a natural inhibitor to the PI3K/AKT pathway. PTEN inhibits the conversion of PIP3 to PIP2. PIP2 limits AKTs' ability to bind to the membrane and therefore decrease activity. The increase in PIP3 results in an accumulation of AKT that is activated by PDK1. AKT activates mTOR by inhibiting TSC2, through phosphorylation. mTOR regulates protein translocation through phosphorylation of S6K1 and eIF4E binding

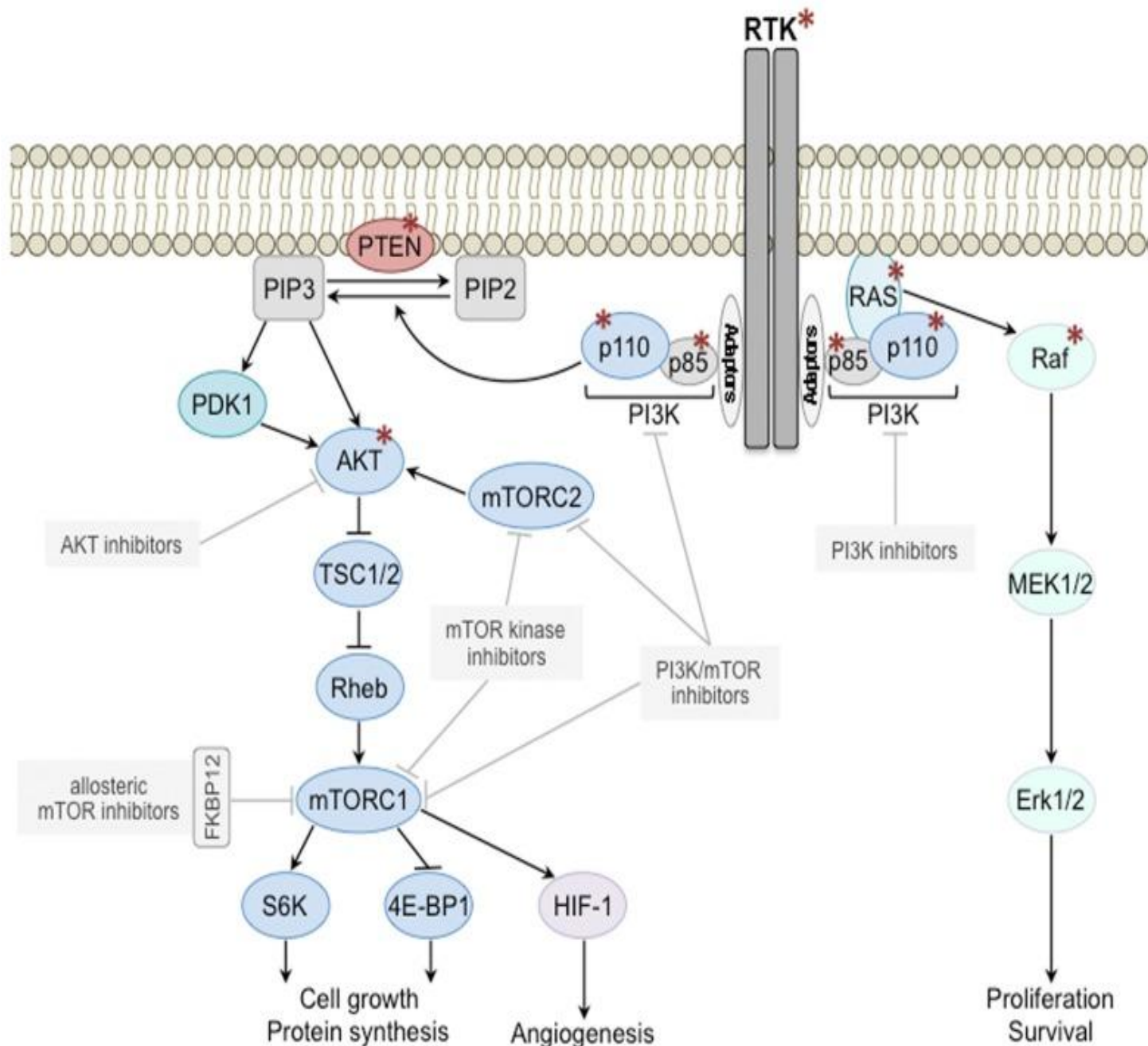
proteins, this activates protein translation and cell survival.<sup>15</sup>

Increases in gene number of the PIK3Ca gene occur particularly in ATC and FTC, this leads to the over expression and activity of the PIK3Ca protein and thus phosphorylation of Akt. This is also associated with the conversion of precancerous epithelial lesions to cancer, distant metastasis and decreased patient survival.<sup>10</sup> Thus RTK (receptor tyrosine kinase) uses the PI3K pathway to promote tumourigenesis and invasiveness of ATC and FTC. Then again this may be used to our advantage to target this genetic event as a potential therapeutic target.<sup>16</sup> RAS is also involved in the PI3K pathway due

to RAS-binding site of p110 catalytic subunits such as that of PI3KCa. *RAS* mutations are associated with the phosphorylation of Akt. *PTEN* methylation is associated with genetic alterations in the PI3K-Akt pathway, such

as hypermethylation of the *PTEN* gene which reduces the inhibitory effect of PTEN. *PTEN* methylation is in fact linked with aggressive cancer.<sup>17</sup>

**Figure 2:** Diagram showing the PI3K pathway. It depicts the different components of the PI3K-AKT and MAPK pathway that are typically affected in cancer. From Weigelt, B. & Downward, J. 2012. Genomic Determinants of PI3K Pathway Inhibitor Response in Cancer. *Front Oncol.*2012;2:109



\*Recurrent genetic (e.g. mutations, amplifications, deletions) and epigenetic (e.g. methylation) alterations

### The Role of BRAF-V600E in the microenvironment

Patients with PTC who also have the BRAF-V600E mutation tend to exhibit a more aggressive clinical behaviour. This might be due to the effect of this mutation on the microenvironment thus effecting proliferation, motility, viability and adhesion. This mutation may also play a part in the conversion of PTC into ATC. Tumorigenesis is affected by the microenvironment of the tumour i.e. non-malignant cells. Genes that are abnormally expressed in cancer may code for receptors and proteins that have an effect on stromal cells such as endothelial cells, macrophages and smooth muscle cells, and on components of the extracellular matrix (ECM). Genes expressed by cells that harbour the BRAF-V600E mutation are for example those of the metallo-proteases (MMPs). MMPs are involved in the breakdown of the ECM and may therefore promote the tumour metastasis. The BRAF-V600E mutation has also been shown to have an effect on thrombospondin-1 (TSP-1). In non-malignant cells, wild-type p53 inhibits angiogenesis by promoting the expression of TSP-1. Reduced expression of TSP-1 is associated with mutated RAS. The role of integrins is to attach the cell to the ECM. Some integrins may act as tumour suppressors and so there is a decreased expression as the cancer progresses, whereas other integrins promote tumour progression such as  $\alpha\beta1$ . ECM components may also interact together to promote cell proliferation, invasiveness and migration. For example, there seems to be a link between integrins and the N-terminal of TSP-1. Fibronectin is over expressed in BRAF-V600E positive cells. It promotes cancer invasiveness through its interaction with integrins. This is due to the constitutive signalling of BRAF-V600E/ERK kinase. Targeting non-malignant components i.e. ECM molecules may provide a novel perspective to treating patients with such cancers.<sup>18</sup>

### Medullary Thyroid Cancer

Medullary thyroid cancer is not as common and accounts for only 3-4% of thyroid cancers. Of these 75% are of the sporadic form while the other 25% account for hereditary cancers. Medullary thyroid cancer occurs in most cases of people who have the MEN 2a syndrome. This is associated with germline mutations in the RET (rearranged during transfection) proto-oncogene, in about 80% of patients this occurs in codon 634 on chromosome 10. Apart from medullary thyroid cancer, MEN 2a is also associated with pheochromocytomas and parathyroid adenomas.<sup>19</sup>

The primary oncogenic event is thought to be a mutation in the receptor tyrosine kinase. In fact therapies target this receptor leading to the development of tyrosine kinase inhibitors (TKI). A receptor tyrosine kinase that is coded for by the *RET* gene, binds to a family of ligands known as glial cell line-derived

neurotrophic factor (GDNF). This in turn leads to the activation of two important pathways that are important in cell differentiation and cell growth, namely the RAS/mitogen-activated protein kinase (MAPK) and the phosphatidylinositol 3' kinase (PI3K)/Akt.

Another receptor that may be involved in MTC is also a tyrosine kinase receptor and is known as the epidermal growth factor receptor (EGFR). The binding of a ligand to this receptor leads to its dimerization and autophosphorylation, which then activates downstream signal pathways. This is involved in only a few MTCs, and was shown to be overexpressed, but not due to gene amplification but as result of polysomes. Therefore drug therapies may target EGFRs and such inhibitors do in fact inhibit the growth of MTC's.

On the other hand vascular endothelial growth factor receptors (VEGFR) are involved in angiogenesis. There are three main VEGFRs: VEGFR-1, VEGFR-2 and VEGFR-3. VEGFR-2 is thought to be involved in tumour metastasis and growth. Tyrosine kinase inhibitors that block VEGFRs have been developed, however their effect is only short lived. Another receptor that may be overexpressed in MTC is the fibroblast growth factor receptor 4 (FGFR4). This receptor is usually involved in cell growth and proliferation and so inhibiting it would halt cell proliferation and therefore tumour growth. The tumour suppressor genes (TSG) retinoblastoma; pRB protein and p53 protein (TP53) are also involved in MTC's. Their interaction with RET results in tumour growth. Mutations and therefore inactivation of both these genes are required in order to prevent apoptosis. Loss of another TSG may result in MTC, PTEN. This may lead to the constitutive activation of the PI3K/Akt pathway.<sup>20</sup>

### Conclusion

The accumulation of genetic alterations of cellular pathways leads to their aberrant signaling. This is what then leads to the development and progression of cancer. These genetic alterations may function as markers and so have a vital role in diagnostics. On the other hand, knowledge of the cellular pathways is useful for therapeutic purposes. Knowledge of the effect of environmental factors may be useful for the prevention of cancer. For example we know that the formation of the *RET/PTC* oncogene is related to exposure of ionizing radiation. The link between endocrine disruptors and the development of cancer in children may further emphasize the importance of prenatal care.

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