

H-Ras alterations in papillary thyroid cancer: a pilot clinicopathological study

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Introduction:

Thyroid cancer is the most common cancer of the endocrine glands in Australia and in other parts of the world. Thyroid cancer occurs primarily in young and middle aged adults, with approximately 122,000 new cases per year worldwide. However, compared with other cancers, little in-depth research has been performed in thyroid cancer in Australia. The main problem that hinders care of thyroid cancer patients is the lack of proper classification of thyroid cancer. This is because thyroid cancer is composed of different histological entities with variable biological behaviour.

More than 95% of thyroid cancer is carcinoma, with papillary thyroid carcinoma accounting for approximately three quarters of thyroid carcinomas. Other common types of thyroid carcinomas include follicular carcinoma, poorly-differentiated carcinoma and undifferentiated carcinoma. Many variants of papillary thyroid carcinoma have been described and some are known to have prognostic significance. Some patients with papillary thyroid carcinoma can also progress to poorly differentiated carcinoma or even to undifferentiated carcinoma of the thyroid. The development pathway taken by a thyroid carcinoma may be influenced by the acquisition of mutations in particular genes (see Figure 1).

As with the majority of cancers, the development of thyroid cancer is influenced by changes to the structure and behaviour of certain genes. There are genetic alterations in three genes that are commonly reported in papillary thyroid carcinoma, namely the BRAF, Ras and RET/PTC oncogenes.

Ras is part of the major growth control pathway in cells. Ras is a family of genes rather than a single gene, all of which are monomeric membrane localised GTPases of roughly 21 kDa each. Ras genes function as signal inducers, taking information directly from receptors and passing that information on to numerous downstream transducers and effectors (see Figure 2). Being a crucial step in the major cellular growth pathway, Ras mutations can have a profound effect on carcinogenesis, affecting cellular growth, differentiation and even cellular mobility and invasion. Many human cancers show Ras mutations. Since the structure of the Ras family is highly conserved, these mutations have similar effects in most Ras family members, tending to fall into the 12, 13 and 61 codons. These mutations eliminate the need for cofactors and lock the Ras genes in an active state. Ras mutations have been known to coexist with other major tranformation mutations mutations at fairly high rates in some cancer types, though this has been found not to be the why this should not be the case in thyroid cancer is not yet fully clear.

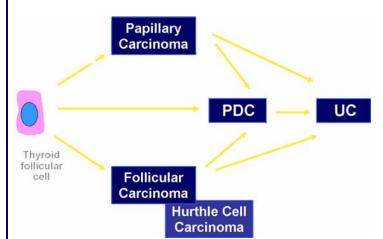
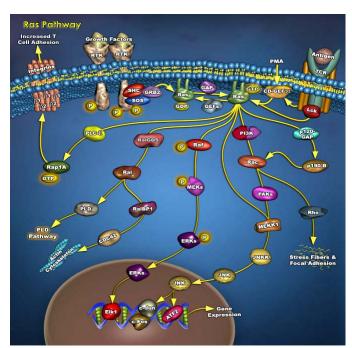


Figure 1: Molecular Alterations in Thyroid Carcinoma

Research has indicated that mutations may influence the development of thyroid carcinomas. Mutations to the BRAF, RAS and RET/PTC genes may result in the papillary carcinoma phenotype, mutations to RAS, along with PAX8-PPARg, may also result in the folliciular phenotype. These may progress into the poorly differentiated (PDC) phenotype, which may also develop directly due to p53 and b-catenin mutations. All phenotypes may further develop into the undifferentiated carcinoma (UC) phenotype, though the mechanisms for this change remain largely unknown.



http://www.ambion.com/tools/pathway/images/Ras%20Pathway.ipg

Figure 2: RAS Gene Pathway.

The RAS gene family is involved in a wide variety of molecular interactions, transferring signals from a number of different sources, including growth factors and immune-stimulating factors. Responses to RAS stimulation include changes to the expression of a variety of genes, as well as effects on cell adhesion, stress, apoptosis and cellular reproduction.

Objective

The aim of the present pilot study is to investigate the clinicopathological roles of mutations in codons 12/13 and 61 of the H-Ras gene in thyroid cancer.

Materials and methods

The formalin fixed paraffin embedded tissues were collected from 19 patients (2 men, 17 women) with a diagnosis of papillary thyroid carcinoma from the Gold Coast Hospital with full ethical approval. The age, gender, clinical presentation, and other clinopathological data was collected in a computerized database. Four of these tissues also had associated lymph node metastases available. DNA was extracted from these tissues using QIAGEN DNA extraction kits. Mutations of the H-Ras gene in codons 12/13 and 61 were determined by polymerase chain reaction, followed by restriction enzyme digestion and visualised on an agarose gel. PCR experiments are summarised in Table 1 and Figures 3 and 4.

Codon 12/13 PCR	111 base pair amplicon
HRAS12_Forward	ATG ACG GAA TAT AAG CTG GT
HRAS12_Reverse	CCT TAT AGT GGG GTC GTA TT
Acil	Digests Wild-type, into 76 and 35 bp
Codon 61 PCR	109 base pair amplicon
HRAS61_Forward	AGG TGG TCA TTG ATG GGG AG
HRAS61_Reverse	AGG AAG CCC TCC CCG GTG CG
HaeIII	Digests Wild-type, into 55 and 54 bp

Table 1: PCR Primers and Digestion Enzyme

Results

After determination of H-Ras mutation status in all tissues, a number of Chi-Square analyses were performed to determine whether or not there were any associations between the H-Ras mutations and clinopathological characteristics. Codon 12/13 mutations were detected in 50% of the tissue population whereas in the codon 61 were noted in one third of the papillary thyroid carcinomas. Three patients showed mutations in both sites of the gene. Chi-square analysis proved negative for all clinopathological characteristics, with the exception of the H-Ras mutation in codon 12/13, which was more often in patients with advanced (stage 3) lesions (p=0.023). Analysis of lymph node metastases was not viable due to low numbers of samples, though it was noted that in these metastases, mutational status was the same, with the exception of one sample which had acquired an additional mutant version of codon 61. Chi-Square results and population genotype status are shown in Tables 2 and 3.

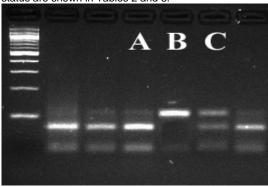


Figure 3: H-RAS Codon 12/13 PCR Digestion Results.

This figure illustrates the results of a PCR and digest of the H-Ras codon 12/13. Lane A shows the digestion products of a wild-type homozygote at 76 and 35 base pairs. Lane B shows the digestion products of a homozygote mutant, at 111 base pairs only. Lane C shows the digestion products of a heterozygote, at 111, 76 and 35 base pairs.

Chi-Square Value	df	Significance
7.552	2	0.023
		40/40 11 1 11

ole 2: Chi-Square Results for Codon 12/13 Mutations in

onclusion:

H-Ras mutations are common in papillary thyroid carcinomas. The results of this study indicate that the possession of mutations in the 12th/13th codon of the H-ras gene correlates with high stage of papillary thyroid carcinoma. This pilot study lacks sufficient numbers to make a definitive statement on the impact of H-ras mutations in papillary thyroid carcinoma. However, these findings imply that codon 12/13 mutations of H-ras may have diagnostic implications in papillary thyroid carcinoma and that further study in this area is warranted. This is particularly important in view of the noted association in this study with H-ras mutations and more aggressive phenotypes.

	Mutants	Heterozygotes	Wild Types
Codon 12/13	9	1	9
Codon 61	6	4	9

Table 3: Genotypes of Tissue Sample Population for H-ras Mutations in Codons 12/13 and 61.

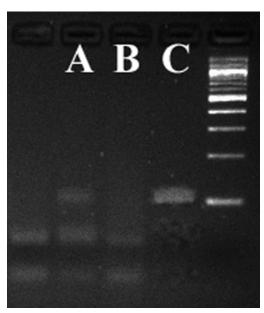


Figure 4: H-RAS Codon 61 PCR Digestion Results.

This figure illustrates the results of a PCR and digest of the H-Ras codon 61. Lane A shows the digestion products of a heterozygote, at 109, 55 and 54 base pairs (primer dimer also visible). Lane B shows the digestion products of a homozygote wild-type 55 and 54 base pairs. Lane C shows the digestion products of a homozygote mutant, at 109 base pairs only.