CASE REPORT

3p25 Aneusomy in Follicular Thyroid Neoplasms: A Report of Three Cases with Review of Literature

CHIA WK[1], ZUBAIDAH Z[2], REENA RAHAYU MZ[3], ROHAIZAK M[4], ASMIATI A[5], RAFIE MK[5], SHARIFAH NA[3]

Departments of [1]Diagnostic Laboratory Services, [3]Pathology and [4]Surgery, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latiff, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia.
[2]Division of Hematology, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia.
Department of [5]Pathology, Putrajaya Hospital, Federal Government Administration Centre, Precint 7, 62250 Putrajaya, Malaysia.

ABSTRAK

Aneusomi merupakan perubahan genetik awal dan suatu ciri utama dalam kebanyakan tumor pejal. Penemuan aneusomi biasanya dikaitkan dengan pesakit kanser dengan prognosis buruk. Penglibatan penyusunan semula gen PAX8-PPARγ dalam tumorigenesis lesi tiroid folikular telah dikaji. Namun begitu, tidak banyak laporan yang melaporkan kehadiran aneusomi gen PPARγ pada lokus 3p25 pada lesi tiroid folikular. Samada kehadiran keabnormalan ini dapat meningkatkan diagnosis, pengkelasan atau prognosis masih tidak dapat ditentukan. Kajian ini melaporkan penemuan aneusomi dalam tiga lesi tiroid folikular [dua karsinoma tiroid folikular (FTC) dan satu kes adenoma sel Hurthle (HCA)] yang menunjukkan kehadiran aneusomi 3p25 menerusi teknik penghibridan in-situ berpendaflur (FISH). Trisomi 3p25 telah ditemui pada satu kes FTC dan satu kes HCA manakala satu kes FTC menunjukkan tetrasomi 3p25. Lesi sel Hurthle berbeza dari segi klinikal dan histologikal daripada neoplasma folikular yang lain. Namun, penemuan aneusomi dalam HCA dan FTC menunjukkan kewujudan pertalian biologi di antara neoplasma sel Hurthle dan folikular. Di samping berkongsi ciri-ciri histologi dengan neoplasma tiroid konvensional, neoplasma sel Hurthle mungkin berkongsi perubahan genetik yang sama di peringkat awal pembentukan tumor folikular.

Kata kunci: aneusomi 3p25, PPARγ, karsinoma tiroid folikular, karsinoma sel Hurthle, penghibridan in-situ berpendaflur

Address for correspondence and reprint requests: Prof. Dr. Sharifah Noor Akmal, Department of Pathology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaakob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur. Tel: 603-9145 5372. Fax: 603-9145 6676. Email: sharifah@ppukm.ukm.edu.my

ABSTRACT

Aneusomy is an early genetic event and a characteristic feature of many solid tumors. It is often associated with poor prognosis in cancer patients. The involvement of *PAX8-PPAR*γ rearrangement in tumorigenesis of follicular thyroid lesions has been widely assessed. However, there were few reports on aneusomy of the *PPAR*γ gene at the 3p25 locus in follicular thyroid lesions. It remains undetermined whether these abnormalities can be translated into improved diagnosis, classification, or outcome prediction. Herein, we report three cases of follicular thyroid neoplasms [two follicular thyroid carcinomas (FTCs) and one Hurthle cell adenoma (HCA)] with 3p25 aneusomy detected by fluorescence *in situ* hybridization (FISH). 3p25 trisomy was observed in one FTC and one HCA while 3p25 tetrasomy was observed in one FTC. Furthermore, all three lesions did not show overexpression of PPARγ protein. Hurthle cell neoplasms (HCN) are distinct clinically and histologically from other follicular thyroid neoplasms (FTN). However, the presence of the aneusomy in HCA and FTC indicates that there could be a biological continuum between the two and chromosomal gains might play an important role in the pathogenesis of these two types of neoplasms. Despite their differences, HCN and FTN may share the same early genetic event in tumour development.

Key words: 3p25 aneusomy, PPARγ, follicular thyroid carcinoma, Hurthle cell adenoma, fluorescence in-situ hybridization

BACKGROUND

Aneuploidy is one of the most frequent genetic abnormalities found in cancer (French et al. 2003; Pihan & Doxsey 2003; Upender et al. 2003; Jonsson et al. 2008; Espadinha et al. 2009). It was known to be the cause of cancer over a century ago, based on Hansemann's observations of asymmetric mitoses in epithelial cancers (Hansemann 1890), which was further proven in 1964, when Boveri showed evidence that aneuploidy in developing sea urchin embryos generates abnormal phenotypes (Boveri 1964).

Aneuploidy, resulting from full or partial aneusomies in which the copy number of entire chromosomes or chromosomal subregions is altered, has been proposed to be an early and genetically destabilizing force in cancer development (French et al. 2003). Increasing aneuploidy is associated with cancer progression and considered as a sign of malignancy, but the molecular mechanisms involved in aneuploidy in cancer remains undefined in the majority of human cancers, particularly in thyroid cancer (French et al. 2003; Banito et al. 2007).

The distribution of aneuploid cells within tumour cells is not uniform, indicating that thyroid cancers may be clonally heterogeneous, which is consistent with the notion that thyroid cancer cells may have chromosomal instability (Ouyang et al. 2002). Possibly due to the varying chromosome

composition, aneuploid cancer cells usually exhibit a wide spectrum of clinical aggressiveness (Isaka et al. 2003). These aneuploid cells could continue to segregate asymmetrically every time they divide in the "chromosome error propagation" process (Carlson et al. 2000). This may explain the association of aneuploid cells in cancer patients with poor prognosis (Ouyang et al. 2002; Barril et al. 2000). There are reports suggesting that follicular carcinomas without PPARγ protein overexpression are more prone to develop distant metastasis, to invade locally, to present with poorly differentiated areas and to persist after surgery, suggesting an association between PPARγ protein underexpression with a less differentiated phenotype (Espadinha et al. 2009; Marques et al. 2004).

Previous studies on thyroid neoplasms reported common aneusomy of chromosome 3, 7, 10, 12 and 17 (Barril et al. 2000; Criado et al. 1995; Belge et al. 1998). Aneusomy of chromosome 7 was also reported in bladder (Wolman et al. 2007) and prostate cancer (Alcaraz et al. 1994), while aneusomy of chromosome 17 was reported in squamous cell carcinoma of the vulva (Carlson et al. 2000), carcinoma of the breast (Wang et al. 2002) and lung (Nakamura et al. 2003). French and co-workers reported 3p25 aneusomy in 29% follicular carcinoma of the thyroid (French et al. 2003). The 3p25 region is associated with *PPARγ* gene, which was widely investigated for its involvement with *PAX8* gene in differentiating follicular thyroid carcinoma (FTC) from its benign counterpart (French et al. 2003; Marques et al. 2004; Kroll et al. 2000; Nikiforova et al. 2002; Dwight et al. 2003; Nikiforova et al. 2003; Cheung et al. 2003; Hibi et al. 2004; Lui et al. 2004; Sahin et al. 2005; Nikiforov 2010; Chia et al. 2010).

Although rare in papillary thyroid carcinomas (PTCs), aneuploidy is a common feature of follicular thyroid adenomas (FTAs) and carcinomas (FTCs) (Espadinha et al. 2009; Banito et al. 2007; Ouyang et al. 2002). Aneuploidy is observed in about 57% FTCs, 10-25% FTAs and 10-22% multinodular hyperplasia (Espadinha et al. 2009; Banito et al. 2007). Besides FTAs and FTCs, one study reported that aneuploidy was also detected in a subset of FTC that shows oncocytic features (Dettori et al. 2003). Some studies have reported that there is a tendency of an increased aneuploidy rate, generally from follicular adenomas to follicular carcinomas and from minimally invasive to widely invasive follicular carcinomas (Grant et al. 1990; Oyama et al. 1994).

World Health Organization (WHO) has categorized Hurthle cell neoplasms (HCN) as a variant of follicular neoplasms, as both neoplasms possess distinct features (Kroll 2004). Among the distinct features that differentiate the HCNs from FTN are the abnormal accumulation of mitochondria in the cytoplasm (Tallini et al. 1999), distinct morphologic features (Kroll 2004), frequent resistance to radio-iodine therapy (Stojadinovic et al. 2002), frequently detected *RET* rearrangements (Chiappetta et al. 2002) and lack of *RAS* mutations and *PPARγ* rearrangements in HCN (Nikiforova et al. 2003).

In this paper, we present three cases of follicular thyroid neoplasms (two FTC and one HCA) with 3p25 aneusomy detected by FISH analysis without PPARγ protein overexpression.

CASE PRESENTATION

Clinicopathological Findings

Case 1 (FC-03)

A 30 year-old lady presented to the surgery clinic of Universiti Kebangsaan Malaysia Medical Centre (UKMMC) with a right thyroid swelling. Fine-needle aspiration cytology (FNAC) yielded cellular smears comprising of thyroid epithelial cells arranged in sheets and follicular pattern. The cells show generally round nuclei with scanty inspissated colloid. The overall features were reported as consistent with a follicular neoplasm. Total thyroidectomy was performed.

Sections from the mass showed a partially encapsulated tumor consisting of neoplastic cells arranged in solid sheets as well as follicular pattern. Collections of inspissated colloid with multifocal areas of haemorrhage and necrosis were also present within the tumor. Generally, the cells display uniform round to oval nuclei with evenly distributed chromatin. Few mitotic figures were evident. A focal area of minimal capsular invasion was noted. However, presence of vascular invasion was not seen. There was no evidence of malignancy in the left thyroid gland and the isthmus. A diagnosis of FTC (minimally invasive) was made.

Case 2 (FC-19)

A 60 year-old lady presented to the surgery clinic, Putrajaya Hospital with a left thyroid swelling. FNA showed cellular smears comprising of neoplastic epithelial cells arranged in microfollicles with scanty to no colloid. Neoplastic cells display nuclear crowding and overlapping. A cytological diagnosis of follicular neoplasm was made.

Total thyroidectomy was performed. Sections from the right lobe and isthmus showed thyroid follicles of varying sizes intersected in areas by fibrous septae. Some of the thyroid follicles appeared dilated. There were areas of haemorrhage and oedema in the intervening stroma. Aggregates of foam cells were also present.

Sections from the left thyroid lobe showed tumour composed of closely packed follicles and trabeculae. The tumour cells were cuboidal with hyperchromatic nuclei. Areas of calcification and haemorrhage were noted but mitotic activity was inconspicuous. In some areas, capsular invasions were seen but no vascular invasion was present. A diagnosis of FTC was made.

Case 3 (FA-18)

A 29 year-old man presented to the surgery clinic, UKMMC with a well-defined neck mass which moved with swallowing. The mass measured 3cm in diameter.

FNAC yielded scanty material. Smears showed a few thyroid epithelial cells arranged in microfollicles as well as singly dispersed cells displaying abundant granular cytoplasm with mild

anisonucleosis. Cytological features were reported as suspicious of a follicular neoplasm, Hurtle Cell type.

The excised specimen consists of two nodular lesions, each measuring 3.5 x 2 x 1.5 cm and 1 x 1.1 x 1.5 cm. The larger nodule revealed a partially encapsulated mass composed of small colloid filled follicles lined by cells with abundant eosinophilic cytoplasm with mild nuclear pleomorphism. Some of the cells showed a trabecular pattern of arrangement. Sections from the smaller nodule showed variable sized colloid filled follicles lined by cuboidal epithelium. The nodules were separated by fibrous bands. No capsular invasion was seen.

A histological diagnosis of follicular adenoma of the thyroid (Hurthle cell type) was made.

Cytogenetic Findings

FISH analysis was performed on the three follicular thyroid neoplasms, i.e. one HCA and two FTCs. Briefly, 3µm thick formalin-fixed paraffin-embedded (FFPE) tissue sections were cut from each of the cases. The sections were deparaffinised in 1 SkipDewax (Insitus Biotechnologies, New Mexico, USA) at 80°C for 30 minutes and digested with 1.5mg/ml pepsin (Sigma-Aldrich Co., St. Louis, USA) at 37°C for 1 hour. The FFPE tissue sections were hybridized with an in-house *PAX8* (green)/ *PPAR*γ (orange) dual colour extra-signal bacterial artificial chromosome-fluorescence *in-situ* hybridization (BAC-FISH) fusion probe assay (Chia et al. 2010) overnight at 37°C. After hybridization, the slides were washed, counterstained, analysed and documented using an epifluorescence microscope system (Applied Spectral Imaging System, Germany). Normal nuclei showed a two orange and two green signal pattern.

3p25 aneusomy was detected in all three follicular thyroid neoplasms. Three orange and two green signal pattern was seen in the trisomy nuclei of one of the FTCs (FC-03). The other case of FTC (FC-19) and the HCA (FA-18) showed four orange and two green signal patterns, indicating 3p25 tetrasomy (Figure 1). All three cases displayed the respective signal patterns in more than 50% of the 200 nuclei analysed. No *PAX8*/*PPAR*γ translocation was observed.

Immunohistochemical Findings

Immunohistochemistry was performed on FFPE tissue sections of all three follicular neoplasms using the monoclonal antibody PPARγ (Santa Cruz Biotechnology, Santa Cruz, CA). Antigen retrieval was performed in 10mM citrate buffer (pH 9.9) at 95°C for 40 minutes before incubating the slides with primary antibody (1:30) for 30 minutes. The slides were then incubated with secondary antibody (ChemMate Dako Envision, HRP rabbit/mouse secondary antibody) and stained with DAB chromogen (DAKO) according to the manufacturer's instruction. No PPARγ overexpression was detected in all three follicular thyroid neoplasms, i.e. two FTCs and one HCA.

DISCUSSION

In this paper, we report three cases of 3p25 aneusomy associated with low PPARγ protein expression in a Hurthle

cell adenoma and two follicular carcinomas of the thyroid. It would be interesting to see whether similar findings have been reported by other authors, as these observations were seen in both benign and malignant lesions of two different entities of follicular thyroid neoplasms.

Aneusomy is often associated with a poor prognosis in cancer patients (Barril et al. 2000) and is considered as an adverse factor in thyroid carcinoma, although the cause of it remained undefined (Banito et al. 2007). Based on the study by Marques et al. (2004), clinically aggressive tumours had lower PPARγ protein expression than less aggressive carcinomas (Marques et al. 2004). This is further supported by the fact that trisomies 7 and 12 in PTCs (which are rarely aneuploid) and FTCs showed a poor clinical outcome (Barril et al. 2000). There are also reports on the association between polysomy (aneusomy or aneuploidy) and high-grade or late stage cancers (Placer et al. 2005; Panani et al. 2004).

Although there were studies suggesting that aneuploidy was associated with genetic instability of many cancers (Banito et al. 2007; Barril et al. 2000; Wolman et al. 2007), it is still not clear why aneuploidy is such a highly variable prognostic marker for different tumours. Lengauer et al. (1998) reported that aneuploidy may not necessarily present with chromosomal instability (Lengauer et al. 1998). While chromosome losses or gains during cell division may trigger apoptosis in normal cells, tumour cells may be protected from apoptosis through a pre-existing mutation (Ouyang et al. 2002). Along this process, the rate of acquisition of chromosomal changes may be normal, and thus aneuploidy develops in the absence of chromosomal instability (Ouyang

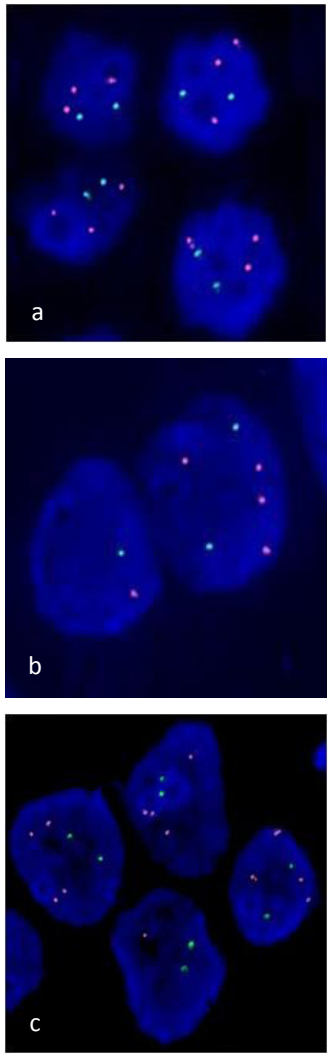

Figure 1: Interphase FISH analysis of (a) FTC (FC-03) showing trisomy nuclei with three orange and two green signal pattern; (b) FTC (FC-19) showing a tetrasomy nucleus with four orange and two green signal pattern; and (c) FTA (Hurthle cell type, FA-18) showing tetrasomy nuclei with four orange and two green signal pattern.

et al. 2002). Meanwhile, Isaka and co-workers (2003) postulated that the wide spectrum of clinical aggressiveness among different aneuploid cancers is due to the existence of different types of cancer aneuploidy created by various molecular defects in gene networks governing the cell cycle, recombination, the repair of double-stranded deoxyribonucleic acid (DNA) breaks and the mitotic checkpoints (Isaka et al. 2003).

None of the three follicular thyroid neoplasms analysed using the in-house *PAX8* (green)/ *PPARγ* (orange) dual color extra-signal BAC-FISH fusion probe assay (Chia et al. 2010) had both 3p25 aneusomy and *PAX8*/*PPARγ* rearrangement, which indicates the involvement of separate independent genetic pathways in early follicular thyroid tumorigenesis. French et al. (2003) suggested either the *PAX8/PPARγ* rearrangement or 3p25 aneuploidy can occur in early follicular carcinomas (French et al. 2003), which is similar to the detection of either RAS mutation or *PPARγ* rearrangement in FTCs (Tallini et al. 1998; Koenig 2010) as well as the detection of either RET rearrangements or NTRK1 rearrangement in PTCs (Pierotti 2001).

Previous cytogenetic studies have reported a high prevalence of trisomies and tetraploidies in FTN (Belge et al. 1998; Hemmer et al. 1998; Roque et al. 1999). We observed presence of aneuploidy in all three cases, both benign and malignant follicular lesions, which is comparable with other previous reports (Banito et al. 2007; Barril et al. 2000; Hemmer et al. 1998; Johannessen et al. 1982). The presence of 3p25 aneusomy in both follicular adenoma and carcinoma of the thyroid support a stepwise adenoma to carcinoma sequence, or indicate the presence of carcinoma in situ. The appearance of 3p25 aneusomy in a HCA reported in this paper further suggests that gain of the 3p25 region is an early event in the development of follicular thyroid neoplasms.

In conclusion, the presence of the aneusomy in both cases of HCA and FTC indicates that there could be a biological continuum between the two neoplasms, and chromosomal gains might play an important role in the pathogenesis of these two types of neoplasms (Chia et al. 2010). Although there has been considerable speculation as to whether Hurthle cell tumours may be clinically and histologically distinct from other follicular thyroid neoplasms, the detection of 3p25 aneusomy in the case of HCA alongside with two FTCs suggests that Hurthle cell neoplasms may be considered a separate follicular thyroid tumour entity but shared the same genetic abnormality in the development of follicular thyroid tumour.

ACKNOWLEDGEMENT

The authors would like to thank Mr. Clarence Ko Ching Huat for his technical advice in the preparation of FISH probe. This project was funded by a research grant from the Malaysian Ministry of Science, Technology and Innovation (MOSTI); grant number 06-02-02-0054 EA250 and approved by UKM ethics committee (UKM FF-031-2005).

REFERENCES

Alcaraz, A., Takahashi, S., Brown, J., Herath, J.F., Bergstralh, E.J., Larson-Keller, J.J., Lieber, M.M. & Jenkins, R.B. 1994. Aneuploidy and aneusomy of chromosome 7 detected by fluorescence in situ hybridization are markers of poor prognosis in prostate cancer. *Cancer Res* **54:**3998-4002.

Banito, A., Pinto, A.E., Espadinha, C., Marques, A.R. & Leite, V. 2007. Aneuploidy and RAS mutations are mutually exclusive events in the development of well-differentiated thyroid follicular tumours. *Clin Endocrinol* **67:**706–711.

Barril, N., Carvalho-Sales, A.B. & Tajara, E.H. 2000. Detection of numerical chromosome anomalies in interphase cells of benign and malignant thyroid lesions using fluorescence in situ hybridization. *Cancer Genet Cytogenet* **117:**50-56.

Belge, G., Roque, L., Soares, J., Bruckmann, S., Thode, B., Fonseca, E., Clode, A., Bartnitzke, S., Castedo, S. & Bullerdiek, J. 1998. Cytogenetic Investigations of 340 Thyroid Hyperplasias and Adenomas Revealing Correlations between Cytogenetic Findings and Histology. *Cancer Genet Cytogenet* **101:**42-48.

Boveri, T. 1964. On multipolar mitosis as a means of analysis of the cell nucleus (originally published 1902). *In* Foundations of Experimental Embryology. Edited by Willier, B.H. & Oppenheimer, J.M. Englewood Cliffs, NJ: Prentice-Hall;74–97.

Carlson, J.A., Healy, K., Tran, T.A., Malfetano, J., Wilson, V.L., Rohwedder, A. & Ross, J.S. 2000. Chromosome 17 aneusomy detected by fluorescence *in situ* hybridization in vulvar squamous cell carcinomas and synchronous vulvar skin. *Am J Pathol* **157(3):**973-983.

Cheung, L., Messina, M., Gill, A., Clarkson, A., Learoyd, D., Delbridge, L., Wentworth, J., Philips, J., Clifton-Bligh, R. & Robinson, B.G. 2003. Detection of the PAX8-PPAR Fusion Oncogene in Both Follicular Thyroid Carcinomas and Adenomas. *J Clin Endocrinol Metab* **88(1):**354-357.

Chia, W.K., Sharifah, N.A., Reena, R.M.Z., Zubaidah, Z., Clarence-Ko, C.H., Rohaizak, M., Naqiyah, I., Srijit, D., Nor Hisham, A., Asmiati, A. & Rafie, M.K. 2010. Fluorescence in situ hybridization analysis using PAX8- and PPARG-specific probes reveals the presence of PAX8-PPARG translocation and 3p25 aneusomy in follicular thyroid neoplasms. *Cancer Genet Cytogenet* **196:**7-13.

Chiappetta, G., Toti, P., Cetta, F., Giuliano, A., Pentimalli, F., Amendola, I., Lazzi, S., Monaco, M., Mazzuchelli, L., Tosi, P., Santoro, M. & Fusco, A. 2002. The RET/PTC oncogene is frequently activated in oncocytic thyroid tumors (Hurthle cell adenomas and carcinomas), but not in oncocytic hyperplastic lesions. *J Clin Endocrinol Metab* **87:**4715-4721.

Criado, B., Barros, A., Suijkerbuijk, R.F., Weghuis, D.O., Seruca, R., Fonseca, E. & Castedo, S. 1995. Detection of numerical alterations for chromosomes 7 and 12 in benign thyroid lesions by *in situ* hybridization. *Am J Pathol* **147:**136-144.

Dettori, T., Frau, D.V., Lai, M.L., Mariotti, S., Uccheddu, A., Daniele, G.M., Tallini, G., Faa, G. & Vanni, R. 2003. Aneuploidy in oncocytic lesions of the thyroid gland: diffuse accumulation of mitochondria within the cell is associated with trisomy 7 and progressive numerical chromosomal alterations. *Genes Chromosomes Cancer* **38:**22-31.

Dwight, T., Thoppe, S.R., Foukakis, T., Lui, W.O., Wallin, G., Hoog, A., Frisk, T., Larsson, C. & Zedenius, J. 2003. Involvement of the PAX8/peroxisome proliferator-activated receptor gamma rearrangement in follicular thyroid tumors. *J Clin Endocrinol Metab* **88:**4440-4445.

Espadinha, C., Pinto, A.E. & Leite, V. 2009. Underexpression of PPARÁ is associated with aneuploidy and lower differentiation of thyroid tumours of follicular origin. *Oncology Reports* **22:**907-913.

French, C.A., Alexander, E.K., Cibas, E.S., Nose, V., Laguette, J., Faquin, W., Garber, J., Moore, Jr.F., Fletcher, J.A., Larsen, P.R. & Kroll, T.G. 2003. Genetic and biological subgroups of low-stage follicular thyroid cancer. *Am J Pathol* **162(4):**1053-1060.

Grant, C.S., Hay, I.D., Ryan, J.J., Bergstralh, E.J., Rainwater, L.M. & Goellner, J.R. 1990. Diagnostic and prognostic utility of flow cytometric DNA measurements in follicular thyroid tumors. *World J Surg* **14:**283–289.

Hansemann, D. 1890. Ueber asymmetrische Zelltheilung in Epithelkrebsen und deren biologische Bedeutung. *Virchows Arch. A Pathol. Anat. Histol* **119:**299–326.

Hemmer, S., Wasenius, V.M., Knuutila, S., Joensuu, H. & Franssila, K. 1998. Comparison of benign and malignant follicular thyroid tumours by comparative genomic hybridization. *Brit J Cancer* **78:**1012–1017.

Hibi, Y., Nagaya, T., Kambe, F., Imai, T., Funahashi, H., Nakao, A. & Seo, H. 2004. Is thyroid follicular cancer in Japanese caused by a specific t(2;3)(q13; p25) translocation generating Pax8-PPARg fusion mRNA? *Endocrine Journal* **51:**361-366.

Isaka, T., Nestor, A.L., Takada, T. & Allison, D.C. 2003. Chromosomal variations within aneuploid cancer lines. *J Histochem Cytochem* **51(10):**1343-1353.

Johannessen, J.V., Sobrinho-Simões, M. & Tangen, K.O. 1982. The diagnostic value of flow cytometric DNA measurements in selected

disorders of the human thyroid. *Am J Clin Pathol* **77:**20–25.

Jonsson, S., Varella-Garcia, M., Miller, Y.E., Wolf, H.J., Byers, T., Braudrick, S., Kiatsimkul, P., Lewis, M., Kennedy, T.C., Keith, R.L., Bjornsson, J., McWilliams, A., Lam, S., Hirsch, F.R. & Franklin, W.A. 2008. Chromosomal Aneusomy in Bronchial High-Grade Lesions Is Associated with Invasive Lung Cancer. *Am J Respir Crit Care Med* **177:**342–347.

Koenig, R.J. 2010. Detection of the PAX8-PPARγ Fusion Protein in Thyroid Tumors. *Clin Chem* **56(3):**331-333

Kroll, T.G., Sarraf, P., Pecciarini, L., Chen, C.J., Elisabetta, M., Spiegelman, B.M. & Fletcher, J.A. 2000. *PAX8-PPARγ1* Fusion Oncogene in Human Thyroid Carcinoma. Science **289:**1357-1360.

Kroll, T.G. 2004. Molecular Events in Follicular Thyroid Tumors. *In* Molecular Basis of Thyroid Cancer. Edited by Farid NR. Boston: Kluwer Academic Publishers; 85-105.

Lengauer, C., Kinzler, K.W. & Vogelstein, B. 1998. Genetic instabilities in human cancers. *Nature* **396:**643-649.

Lui, W.O., Foukakis, T., Liden, J., Thoppe, S.R., Dwight, T., Hoog, A., Zedenius, J., Wallin, G., Reimers, M. & Larsson, C. 2004. Expression profiling reveals a distinct transcription signature in follicular thyroid carcinomas with a PAX8-PPARγ fusion oncogene. *Oncogene* 1-10.

Marques, A.R., Espadinha, C., Frias, M.J., Roque, L., Catarino, A.L., Sobrinho, L.G. & Leite, V. 2004. Underexpression of peroxisome proliferator-activated receptor (PPAR) gamma in PAX8/PPAR gamma-negative thyroid tumours. *Brit J Cancer* **91:**732-738.

Nakamura, H., Saji, H., Idiris, A., Kawasaki, N., Hosaka, M., Ogata, A., Saijo, T. & Kato, H. 2003. Chromosomal Instability Detected by Fluorescence in Situ Hybridization in Surgical Specimens of Non-Small Cell Lung Cancer Is Associated with Poor Survival. *Clin Cancer Res* **9:**2294-2299.

Nikiforova, M.N., Biddinger, P.W., Caudill, C.M., Kroll, T.G. & Nikiforov, Y.E. 2002. PAX8-PPARgamma rearrangement in thyroid tumors: RT-PCR and immunohistochemical analyses. *Am J Surg Pathol* **26:**1016-1023.

Nikiforova, M.N., Lynch, R.A., Biddinger, P.W., Alexander, E.K., Dorn II, G.W., Tallini, G., Kroll, T.G. & Nikiforov, Y.E. 2003. RAS point mutations and PAX8-PPAR gamma rearrangement in thyroid tumors: evidence for distinct molecular pathways in thyroid follicular carcinoma. *J Clin Endocrinol Metab* **88:**2318-2326.

Nikiforov, Y.E. 2010. Recent Developments in the Molecular Biology of the Thyroid. *In* Lloyd RV (ed). Endocrine Pathology. Springer: New York; 237-260.

Ouyang, B., Knauf, J.A., Ain, K., Nacev, B. & Fagin, J.A. 2002. Mechanisms of aneuploidy in thyroid cancer cell lines and tissues: evidence for mitotic checkpoint dysfunction without mutations in BUB1 and BUBR1. *Clin Endocrinol* **56:**341-350.

Oyama, T., Vickery, A.L.Jr., Preffer, F.I. & Colvin, R.B. 1994. A comparative study of flow cytometry and histopathologic findings in thyroid follicular carcinomas and adenomas. *Hum Pathol* **25:**271–275

Panani, A.D., Babanaraki, A., Malianga, E. & Roussos, C. 2004. Numerical aberrations of chromosomes 9 and 11 detected by FISH in Greek bladder cancer patients. *Anticancer Res* **24:**3857-3862.

Pihan, G. & Doxsey, S.J. 2003. Mutations and aneuploidy: Co-conspirators in cancer? *Cancer Cell* **4:**89-94.

Pierotti, M.A. 2001. Chromosomal rearrangements in thyroid carcinomas: a recombination or death dilemma. *Cancer Lett* **166:**1–7

Placer, J., Espinet, B., Salido, M., Sole, F. & Gelabert-Mas, A. 2005. Correlation between histologic findings and cytogenetic abnormalities in bladder carcinoma: a FISH story. *Urology* **65:**913-918.

Roque, L., Serpa, A., Clode, A., Castedo, S. & Soares, J. 1999. Significance of trisomy 7 and 12 in thyroid lesions with follicular differentiation: a cytogenetic and *in situ* hybridization study. *Lab Invest* **79:**369–378.

Sahin, M., Allard, B.L., Yates, M., Powell, J.G., Wang, X-L., Hay, I.D., Zhao, Y., Goellner, J.R., Sebo, T.J., Grebe, S.K.G., Eberhardt, N.L. & McIver, B. 2005. PPARγ Staining as a Surrogate for *PAX8/PPARγ* Fusion Oncogene Expression in Follicular Neoplasms: Clinicopathological Correlation and Histopathological Diagnostic Value. *J Clin Endocrinol Metab* **90(1):**463-468.

Stojadinovic, A., Hoos, A., Ghossein, R.A., Urist, M.J., Leung, D.H., Spiro, R.H., Shah, J.P., Brennan, M.F., Singh, B. & Shaha, A.R. 2002. Hurthle cell carcinoma: a 60-year experience. *Ann Surg Onco.* **9:**197-203.

Tallini, G., Santoro, M., Helie, M., Carlomagno, F., Salvatore, G., Chiappetta, G., Carcangiu, M.L. & Fusco, A. 1998. RET/PTC oncogene activation defines a subset of papillary thyroid carcinomas lacking evidence of progression to poorly differentiated or undifferentiated tumor phenotypes. *Clin Cancer Res* **4**(2)**:**287–294.

Tallini, G., Hsueh, A., Liu, S., Garcia-Rostan, G., Speicher, M.R. & Ward, D.C. 1999. Frequent chromosomal DNA unbalance in thyroid oncocytic (Hurthle cell) neoplasms detected by comparative genomic hybridization. *Lab Invest* **79:**547-555.

Upender, M.B., Habermann, J.K., McShane, L.M., Korn, E.L., Barrett, J.C., Difilippantonio, M.J. & Ried, T. 2004. Chromosome Transfer Induced Aneuploidy Results in Complex Dysregulation of the Cellular Transcriptome in Immortalized and Cancer Cells. *Cancer Res* **64:**6941–6949.

Wang, S., Saboorian, M.H., Frenkel, E.P., Haley, B.B., Siddiqui, M.T., Gokaslan, S., Hynan, L. & Ashfaq, R. 2002. Aneusomy 17 in Breast Cancer: Its Role in HER-2/neu Protein Expression and Implication for Clinical Assessment of HER-2/neu Status. *Mod Pathol* **15(2):**137-145.

Wolman, S.R., Goldman, B., Slovak, M.L., Tangen, C., Persons, D.L. & Wood, D. 2007. Aneusomy for detection of bladder cancer recurrence: a Southwest Oncology Group study. *Cancer Genet Cytogenet* **176:**22-27.