LETTER TO THE EDITOR

Unique landscape of widespread chromosomal losses in Hürthle cell carcinoma

In their back-to-back published articles ‘Integrated Genomic Analysis of Hürthle Cell Cancer Reveals Oncogenic Drivers, Recurrent Mitochondrial Mutations, and Unique Chromosomal Landscapes’ and ‘Widespread Chromosomal Losses and Mitochondrial DNA Alterations as Genetic Drivers in Hürthle Cell Carcinoma’ [Ganly *et al.* \(2018\)](#) and [Gopal *et al.* \(2018\)](#), respectively, defined alterations in oncogenic drivers and mitochondrial DNA mutations complex I (mtDNA), including the widespread whole-chromosome losses in oncocytic or Hürthle cell thyroid carcinoma (HCC). This comprehensive analysis further unravels the unique features of HCC, either minimal or widely invasive in character and confirms previous work published on mtDNA mutations as well as the widespread loss of entire chromosomes ([Bonora *et al.* 2006](#), [Gasparre *et al.* 2007](#), [Corver *et al.* 2012, 2014](#)).

In this letter, we want to address the questions left unanswered in both manuscripts. A possible mechanism behind the observed whole-chromosome losses as well as the possible occurrence of such chromosomal losses in benign Hürthle cell lesions (adenomas) was not discussed or studied. Thirdly, [Ganly *et al.* \(2018\)](#) and [Gopal *et al.* \(2018\)](#) both use the term uniparental disomy of which the use, to our opinion, should be restricted to single chromosomal events rather than the widespread loss of many entire chromosomes as observed in HCC. Finally, a significant number of the HCCs studied in these papers do not show mtDNA mutations, confirming our earlier finding ([Corver *et al.* 2014](#)) and pointing to another origin of the mitochondrial expansion and the widespread loss of entire chromosomes so typically for oncocytic lesions.

We described a range of whole-chromosome copy number changes observed in Hürthle cell adenomas (HCA) ([Corver *et al.* 2017](#)). The occurrence of genetic alterations in Hürthle cell hyperplasia and HCA was also studied by [Nikiforova *et al.*](#) in their evaluation of the ThyroSeq v3 genomic classifier for preoperative cytological analysis of thyroid nodules ([Nikiforova *et al.* 2018](#)). Chromosomal copy number alterations were observed in 33% of the

Hürthle cell adenomas but not in Hürthle cell hyperplasia. No specific chromosomal information was given. From our observations, we developed a model for HCC (annotated at the time as FTC-OV by us) based on whole-chromosome instability (w-CIN) and step-wise loss of whole chromosomes during tumor progression, thereby leading to near-homozygous genomes (NHGs). We found support that excessive amounts of reactive oxygen species (ROS) likely initiate the process ([Corver *et al.* 2017](#)).

One of characteristics of HCC is the abundance of dysfunctional mitochondria producing elevated levels of ROS. Without significant DNA damage, ROS can activate the ATM-CHK2 pathway ([Alexander *et al.* 2010](#), [Guo *et al.* 2010](#)). Simultaneously, ATM-CHK2 activation causes increased kinetochore-microtubule stability during mitosis causing lagging chromosomes ([Bakhoum *et al.* 2014](#)). We studied the number of chromosome missegregations in cell line models for thyroid cancer with and without NHG. Amongst these were [XTC.UC1](#) cells, the only official model for HCC and FTC-236 cells, both showing a NHG highly comparable to the NHGs found in clinical HCC, including retention of chromosome 7. FTC-236 cells (including FTC-133 and FTC-238, derived from different metastasis from the same patient) were previously reported to originate from a follicular thyroid cancer and not from HCC ([Demeure *et al.* 1992](#)). Chromosome missegregations were substantially higher in [XTC.UC1](#) and in FTC-236 than the other thyroid cancer cell lines that did not show a NHG and were significantly reduced after ROS scavenger experiments. From FTC-236 cells we were able to isolate subclones all showing a unique pattern of a NHG pointing to continuing w-CIN in this cell line model. One minimal-invasive HCC showed whole-chromosome imbalances, which can be attributed to intra-tumor heterogeneity caused by an admixture of populations: one heterozygous (two copies) and one homozygous (one copy) for the involved chromosomes. Strikingly, the affected chromosomes (1, 2, 8, 9, 18 and 22) present in variant frequencies in HCA samples showed to be homozygous in all HCCs analyzed, suggesting selection for the loss of these

chromosomes during clonal outgrowth. Furthermore, from one case we obtained two samples from the primary tumor that clearly displayed different NHGs, again demonstrating w-CIN leading to intra-tumor heterogeneity.

In the context of the proposed progression model of HCC, flow cytometric DNA content analysis of HCA and HCC revealed that a near-haploid state precedes the endoreduplicated and aneuploid state caused by genome doubling (Corver *et al.* 2017). Genome doubling is a common feature of carcinomas. For example, studies on DNA content analysis clearly revealed a bi-model distribution of the DNA content of many carcinomas in which a step of tetraploidization (endoreduplication of a DNA diploid population) precedes aneuploidization, for example, in Barrett's esophagus and breast cancer (Devilee & Cornelisse 1994, Galipeau *et al.* 1996). Thus, the copy neutral LOH of many entire chromosomes found in HCC is the consequence of genome doubling after a process of near-haploidization rather than uniparental disomy, as proposed by Ganly *et al.* and Gopal *et al.*, which involves two copies of a chromosome as the consequence of a meiosis I or II error (Robinson 2000). The proposed mechanism seems to operate in other types of cancers showing oncocyctic subtypes with a NHG, like adrenal cortical cancer, isolated parathyroid cancers (Corver *et al.* 2014), acute lymphoblastic leukemia (Holmfeldt *et al.* 2013) and a subset of chondrosarcoma (Bovee *et al.* 2000).

Whole chromosome genomic alterations can also have unexplained features in thyroid cancer. We found exceptional cases of non-oncocyctic follicular thyroid cancers with widespread whole chromosome gains that cannot be explained by genome endoreduplication and additional alterations of whole chromosomes. Figure 1 is a clear example of DNA aneuploid FTC with a DNA index of 1.26 with widespread whole-chromosome copy number changes, foremost with whole chromosomal gains and only one (chromosome 3) showing copy-neutral LOH.

Better understanding of the molecular mechanisms underlying the widespread w-CIN might lead to new therapeutic options highly needed for these types of aggressive cancers. Furthermore, it would be interesting to study the exact role of mtDNA mutations in HCC. Also by Ganly *et al.* and Gopal *et al.*, it was proposed that complex I mtDNA mutation plays a pivotal role in the development and progression of HCC (Gasparre *et al.* 2007). Conversely, we and Ganly *et al.* and Gopal *et al.* also showed that many remaining HCCs with NHG do not carry disruptive (or damaging) complex I mtDNA mutations. Apparently, alternative explanations should be found. One avenue that might be further explored is the hypothesis that extensive mitochondrial hyperplasia found in HCC is driven by a mitochondrial coupling defect (Savagner *et al.* 2001). The latter might give clues for alternative explanations for the observed NHG.

Figure 1

A DNA aneuploid follicular thyroid carcinoma (FTC) with widespread whole-chromosome copy number changes. (A) H&E section (40x) from a formalin-fixed, paraffin-embedded specimen of the thyroid tumor. Punches from the same tissue block were used for preparing a cell suspension which was labeled for keratin (FITC), vimentin (APC) and DNA (PI) and analyzed on an LSRII (BD Biosciences) flow cytometer (Corver *et al.* 2005). (B) DNA histogram of the vimentin-positive stromal fraction (green, internal reference), (C) keratin vs vimentin dot plot. Note that most of the carcinoma cells show vimentin co-expression, (D) DNA histogram of the keratin-positive carcinoma fraction (red), (E) overlay DNA histograms stromal fraction and carcinoma fraction. The DNA index (DI) was 1.26 (ModFit analysis, Verity Software House). (F) (inlay) negative control showing green and deep red background fluorescence. (G) DNA was extracted from a corresponding frozen section of the same FTC and prepared for single nucleotide polymorphism analysis (cytoSNP, Illumina). Note that chromosome 3 shows copy-neutral LOH (AA). Chromosomes 2, 6, 8, 10, 12, 13, 15, 17 and 20 are heterozygous with two copies (AB). Chromosome 14 shows four copies (AABB). The other chromosomes are imbalanced with three copies (AAB).

Willem E Corver
Hans Morreau

Department of Pathology, Leiden University Medical
Center, Leiden, The Netherlands

(Correspondence should be addressed to W E Corver and
H Morreau: w.e.corver@lumc.nl and j.morreau@lumc.nl)

Declaration of interest

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