Editorial

Molecular changes are infrequent in thymic carcinomas but might represent targetable mutations

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Thymic carcinomas are in general the most aggressive thymic epithelial tumors (TET) with a median survival of only 2.0 to 3.6 years (1-3). Disease recurrence and metastases are not uncommon and are described in 41% to 50% of patients (1,4). Moreover, thymic carcinomas often present as a large, locally aggressive lesion precluding complete resection. Therefore, many of these patients are treated with neoadjuvant therapy which may or may not result in resectability of the tumor. Hence, additional treatment options are needed. Because these tumors are often already of high stage at time of diagnosis, commonly only biopsy material is available for studies. Moreover, thymic carcinomas are very rare and even less common than thymomas which have an incidence of only 1.5 per million people per year (5,6). The paucity of these lesions hampers large, meaningful studies and in general requires multi-institutional, global efforts. In addition, some molecular studies can only be performed using frozen tissue which is usually not available from biopsy material and only occasionally collected from resection specimens.

The heterogeneity of thymic carcinomas poses another challenge of their study. While squamous cell carcinomas are most common comprising 29% to 67% of all thymic carcinomas, other subtypes include basaloid carcinoma, clear cell carcinoma, sarcomatoid carcinoma, adenocarcinoma, undifferentiated carcinoma, mucoepidermoid carcinoma, NUT carcinoma, and lymphoepithelioma-like carcinoma, amongst others as summarized by the 2015 WHO classification (1,7). In rare occasions, multiple subtypes of carcinoma might occur within one thymic carcinoma.

The paucity of thymic carcinomas together with their morphologic heterogeneity and usually only limited available material make molecular studies of these tumors difficult. Therefore, only small case studies are available hampering the development of molecularly targeted drugs. However, given the often aggressive nature of thymic carcinomas, the development and investigation of potential targeted therapy for these tumors is of great importance.

For some time activating mutation of the tyrosine kinase KIT was the only known targetable alteration in thymic carcinomas, present in 6–12 % of cases (8-11). Recently, more sophisticated molecular techniques including next generation sequencing (NGS) led to more in depth studies of molecular changes in thymic carcinomas. For instance, Moreira *et al.* performed paired tissue (tumor and matched normal) analysis on 15 thymic carcinomas using exon capture of 275 cancer-related genes, followed by deep coverage NGS (12). The authors found non-silent somatic mutations in 12 of 15 (80%) thymic carcinomas even though the overall rate of mutations was low with a median of one mutation per tumor (range, 0–26). Most common were mutations in the tumor suppressor gene *TP53*, which were identified in 30% of cases. Other studies also showed that *TP53* mutations are relatively common in thymic carcinomas ranging between 7.5% and 26% (10,11,13). Moreira *et al.* further showed that p53 overexpression correlates with *TP53* mutation and a higher rate of recurrence and death of disease compared to carcinoma with normal p53 expression (12). Wang *et al.* also showed that *TP53* mutations in TET are associated with worse survival, although a subset analysis of thymic carcinomas was not performed (11). However, overall, these findings

suggest that tumors with *TP53* mutation may behave more aggressively (12). Mutations were also identified in *KDM6A* (25%), *SMAD4* (17%), *CYLD* (9–17%), *SETD2* (9–17%), *MLL3* (17%), *MLL2* (17%), *BAP1* (13%), *DNMT3A* (6%), *FGFR3* (6%), *TET2* (4%), *DCC* (4%), *ASXL1* (4%), *WT1* (4%), *SMARCA4* (4%), *BRCA2* (4%), *KRAS* (3.8%), *FBXW7* (3.8%), *ALK* (3%), *ATM* (3%), *ERBB4* (3%) and *NRAS* (2–3%) (10-13). Concurrent mutations were only rarely identified and included *TP53* and *KRAS*, and *TP53* and *FBXW7* (11).

Interestingly, in the study by Moreira *et al.* the highest mutation rate (n=26) was identified in an undifferentiated carcinoma while all other thymic carcinomas in that study were of squamous cell carcinoma subtype and harbored only between one and five mutations in 11 tumors (12).

Recently, a missense mutation in *GTF2I* has been identified in TET, most commonly in type A and AB thymoma (74–87%) and less common in types B1, and B2 (20–32%) and B3 thymoma (10–21%) (14,15). Despite its common occurrence in thymoma, *GTF2I* mutations have only been identified in 8% of thymic carcinomas (14,15). Although the clinical relevance of this mutation has not been fully elucidated evidence suggests that *GTF2I* mutations might be associated with a better prognosis in TET (14,15). For instance, Feng *et al.* showed that *GTF2I* mutation is an independent prognostic parameter being associated with more indolent tumors (14). However, that study did not have the power to perform a statistical subgroup outcome analysis for thymic carcinomas given the low number of mutation-carrying tumors. *GTF2I* mutations in TET have all been identified to affect the same nucleotide on chromosome 7q11.23 causing alterations in transcription factor IIi (TFII-I) isoforms. TFII-I is a signal-induced multifunctional transcription factor that appears to be involved in the regulation of genes that are important for cell growth, cell cycle and cell division (16). Although currently very little is known about the role of TFII-I in human tumors, because it is thought to control cell proliferation and cell cycle *in vitro*, a role of TFII-I in cancer cell growth control has been hypothesized.

Petrini *et al.* identified homozygous 9p21.3 copy number loss (attributed to *CDKN2A/B* loci) in 2 (of 7, 28.6%) thymic carcinomas and 2 (of 20, 10%) WHO type B3 thymomas (17). Similarly, Enkner *et al.* identified *CDKN2A* mutations in 11% of thymic carcinomas (10). The homozygous 9p21.3 copy number loss was attributed to *cyclin-dependent kinase inhibitor 2A/B (CDKN2A/B)* loci. Although in that study copy number loss of *CDKN2A/B* was associated with worse disease related survival and time to progression, subset analysis for thymic carcinomas was not performed likely because of the low power of the study. Cases with copy number loss of *CDKN2A/B* lacked expression of p16 as *CDKN2A* encodes p16 (INK4a) (18). p16 protein acts through inhibition of CDK4 and CDK6. It has a negative regulatory effect on cell cycle progression. Inactivating mutations lead to alteration of this inhibitory function permitting inappropriate progression through the cell cycle resulting in uncontrolled tumor cell proliferation. Subsequently, in a study of 26 thymic carcinomas Aesif *et al.* showed loss of p16 expression in 12 (46%) tumors which was associated with worse recurrence/metastasis free survival independent of resection status and T-stage (19). In a subset analysis of 15 thymic squamous cell carcinoma loss of p16 expression, identified in five (33.3%) cases, was associated with worse recurrence/metastasis free survival and overall survival. In addition, patients with thymic carcinoma that had loss of p16 expression were younger. Overall, 18% of thymic carcinomas showed homozygous *CDKN2A* deletion which, similarly to the study by Petrini *et al.*, correlated with loss of p16 expression. The authors concluded that loss of p16 expression and homozygous deletion of *CDKN2A* are promising prognostic biomarkers in thymic carcinomas. Furthermore, a subset of thymic carcinomas might respond to CDK4/6 inhibitors. However, further functional studies are needed.

EGFR gene and its downstream pathway has also been investigated in thymic carcinomas. In a study using RT-PCR and direct sequencing to assess the mutations in the *EGFR* downstream pathway of 61 thymic carcinomas, gene mutations were identified in 7 patients (11.5%) (20). Most mutations in that pathway were identified in the *KRAS* gene (n=4); other mutations were found in the *BRAF*, *PIK3CA* and *EGFR* genes (n=1, each). Again, mutations in *EGFR* and its downstream pathway are not very common. However, even if these findings are infrequent they raise the possibility of potential usefulness of targeted therapy in these patients. Another study using NGS with a panel that analyzed 1,800 mutational hotspots and targeted regions in 22 genes including *EGFR*, *KRAS*, *BRAF*, *PIK3CA*, *TP53*, *SMAD4* amongst others revealed only a mutation in the *PIK3CA* gene in 1 (of 15, 6.7%) thymic carcinoma further confirming that gene mutations are rare in these tumors (21).

Although gene mutations appear to be infrequent in thymic carcinomas certain mutations are potentially targetable and therefore are important to study further. Alberobello *et al.* using standard biomarker analysis and

NGS and functional assays characterized MP57, a thymic carcinoma cell line. The authors identified mutations in *PIK3R2*, *TP53*, *TAF1*, *CSNK2A3*, *SGK223*, and *TTN* genes in the cell line and also in the corresponding primary tumor (22). The mutation in *PIK3R2* appeared to be of particular interest to the authors as it represents a regulatory subunit of *PI3K*. Further analysis identified different mutations in multiple *PI3K* subunits in three other primary thymic carcinomas (although it is not clear how many thymic carcinomas were tested in total) including two catalytic subunits (*PIK3CA* and *PIK3CG)* and another regulatory subunit (*PIK3R4*). Moreover, inhibiting *PI3K* with GDC0941, a pan-PI3K inhibitor, resulted in *in vitro* antitumor activities in the thymic carcinoma cell line.

In conclusion, although mutations are rather uncommon in thymic carcinomas, multiple gene mutation analysis might reveal occasional targetable mutation(s) in a small subset of thymic carcinomas. However, large multi-institutional studies of well-characterized thymic carcinomas would be important to identify additional targetable mutations, to characterize their clinical significance and to correlate their occurrence to morphologic subtypes of thymic carcinomas. As prospective clinical trials limited to thymic carcinomas will be challenging to perform given the paucity of these tumors, these patients might be enrolled into other trials based on molecular findings in their tumors (23).

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