Molecular aspects of post-Chornobyl and sporadic papillary thyroid carcinoma

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Abstract

Papillary thyroid carcinoma (PTC) exhibits various molecular abnormalities, both when sporadic and radiation-related. PTC is still diagnosed in adult individuals who were younger than 18 years at the time of the Chornobyl accident in 1986 and lived within the contaminated area. The preoperative diagnosis of PTC is based on ultrasound-guided fine needle aspiration cytology (FNAC), which is highly informative in up to 90% of biopsies. FNAC is not informative for the discrimination of follicular thyroid carcinoma (FTC) from follicular thyroid adenoma (FTA). Moreover, FNAC is often unreliable for diagnosis of cystic PTC due to its common presentation as a mural nodule in a cystic mass. In case of cystic PTC, biopsy sometimes reveals a cystic fluid containing insufficient amount of representative cells for cytology.

In this work, PTC was characterized in relation to irradiation from radioactivity at childhood. Possible preoperative diagnostic markers for discrimination between PTC and other follicular thyroid neoplasms were identified, and their validity was tested.

In Study I molecular, genetic and clinical characteristics in 70 post-Chornobyl PTCs were investigated. A common BRAF 1799T>A mutation was detected in 26 cases, overrepresentation of RET/PTC1 in 20 whereas RET/PTC3 was found in 4 cases. BRAF mutation was observed 3.5 times less frequent in the PTC accompanied by chronic lymphocytic thyroiditis (PTC/CLT) as compared to PTC only (12% vs. 44%). Greater expression of cyclin A was observed in PTC ≥ 2 cm as compared to PTC < 2 cm (1.2% vs. 0.6%). In conclusion, BRAF mutation and RET/PTC1 rearrangement as well as other molecular features of adult post-Chornobyl PTC were partly overlapping with other reported PTC cohorts.

In Study II the SELDI-TOF mass spectrometry method was applied for PTC, FTC, FTA and normal thyroid tissue (NT). Significant overexpression of the protein S100A6 was identified in PTC as compared to FTC, FTA and NT (p < 0.05). This result was verified both by Western blot (WB), using the same samples, and by IHC in these and additionally in the PTC samples investigated in Study I. Moreover, the presence of two post-translational modifications of S100A6 was observed and verified by LC-MS/MS. S100A6 expression is strongly associated with PTC, and can therefore be tested for discrimination between follicular thyroid tumors and PTC.

In Study III a two dimensional gel electrophoresis followed by MALDI-TOF mass spectrometry for proteomic profiling of PTC, FTC and FTA was performed. 25 protein spots showing significantly different expression between studied groups were identified. Of these, 9 protein spots were selected for further analyses by WB using the initially studied samples and by IHC using these as well as samples from Study I. The findings suggest additional proteins to be deregulated in thyroid tumors, and their clinical significance can now be further studied.

In Study IV preoperative diagnostic markers for PTC in cystic lesions were identified by applying LC-MS/MS method. Out of all 1581 identified proteins, annexin A3 (ANXA3), carboxymethylenebutenolidase homolog (CMBL) cytokeratin 19 (CK-19) and S100A13 were selected for validation by IHC and WB. ANXA3 and CMBL showed overexpression in both controls and PTCs, whereas S100A13 and CK-19 were up-regulated in PTC only (p < 0.05), suggesting their possible role for discrimination between cystic PTC and benign thyroid cysts.

Keywords: papillary thyroid carcinoma, Chornobyl, proteomics profiling, BRAF mutation, RET/PTC, S100A6, S100A13, cytokeratin 19.

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