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Abstract of the PhD thesis:

Morphological, immunophenotypic and molecular aspects of gastric carcinomas

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Gastric cancer is one of the most aggressive tumors, ranking 5th globally in terms of incidence and 4th in terms of mortality. The most common type of gastric cancer (over 90%) is gastric carcinoma (GC). GC is known to be one of the most heterogeneous tumors. This heterogeneity is evident both in terms of histological aspects, but especially in terms of genetic and molecular characteristics of the tumors, which makes it difficult to implement a universal classification system. Moreover, the major phenotypic diversity present within the same tumor negatively influences the efficacy of cancer therapy. Therefore, this feature of GC is one of the most important underlying causes of its transformation into a real global health problem.

The aim of this thesis was to perform a clinicopathological analysis of GC cases diagnosed in the Emergency County Clinical Hospital of Targu Mures, in the period 2017-2021, testing hypotheses validated immunohistochemically (IHC) and by fluorescence in-situ hybridization (FISH). We hypothesized that the phenotypic divergence of these tumors requires the implementation of additional biomarkers with diagnostic and/or prognostic role, with acceptable specificity and sensitivity.

In the first chapter, we tested the immunophenotypic heterogeneity of HER2 expression using two different clones of the same antibody. We also confirmed the extent of HER2 expression by FISH technique. Of the total 93 patients included in this study, 22.58% had tumors with at least focal HER2 positivity and at least 1+, independent of the clone used. 7.53% showed 3+ (positive) immunostaining using the polyclonal antibody, and when using the CB11 clone, cases with 3+ immunostaining accounted for 6.45%. In 7.53% of cases, HER2 immunostaining was 2+ (equivocal) independent of the clone used. The equivocal group was represented by the same number of cases due to a slight tendency of HER2 undescoring in the case of CB11 clone, compared to polyclonal antibody: one 3+ case (using polyclonal antibody) was classified as 2+ (using CB11 clone), respectively one 2+ case (using polylconal antibody) was classified as 1+ (using CB11 clone). Category 1+ (negative) was represented by 7.53% of cases with the polyclonal antibody and 8.6% of cases with CB11 clone, respectively. All 3+ cases were confirmed as amplified by FISH, respectively all 1+ cases were confirmed as non-amplified. More than half of the cases with equivocal, 2+ immunostaining, showed gene amplification.

HER2 analysis on multiple sections from the same case demonstrated marked intratumoral heterogeneity, both between different areas of the tumors and between tumor cells within the same gland. In gastric cancer, *HER2* gene is predominantly amplified in well- or moderately differentiated adenocarcinomas. Establishing a diagnosis of HER2-negative GC by IHC requires analysis of at least three or four tumor sections, taken from different foci. FISH analysis of *HER2* gene status is indicated in all cases showing type 3+, even if the positivity is encountered in less than 5% of cells.

Both clones of anti-HER2 antibodies tested in the present thesis can be used in daily practice, showing superimposable results in more than 90% of cases. For FISH assessment, it is reccomended the to use a section

where a 3+ focus has been demonstrated, regardless of its size. In GC, *HER2* gene amplification is an independent indicator of negative prognosis.

The second chapter had two main themes, one focused on the implications of VSIG1 in gastric carcinogenesis annd epithelial-mesenchymal transition (EMT), and the second, on the way of expression and interaction of mucins with other molecules in GC. We aimed to highlight the possible prognostic or predictive impact of mucin phenotype in CG, and to elucidate the molecular networks modified as a result of altered metabolism of these molecules. Also, based on the hypothesis that there are differences between the way of VSIG1 expression in the tumor center and its periphery/tumor invasive front, we analysed IHC expression in these two locations and classified GC according to its way of variation, performing on these considerations a stratification of patients.

Thus, we divided the cases into three major categories: homologous type I (membrane immunstaining in both tumor center and invasive front) – 20 cases, homologous type II (null reaction in both tumor center and invasive front) – 53 cases, and heterologous subtype, with two subdivisions (type A – membrane immunostaining in the tumor center, with cytoplasmic translocation in invasive front – 5 cases, and type B - cytoplasmic immunostaining in the tumor center with loss of VSIG1 in the invasive front – 15 cases). Of these VSIG1 types, the lowest survival rate was recorded for the heterologous subtype. Also, the same type of immunostaining was associated with EMT, demonstrated by loss of E-cadherin expression and nuclear translocation of β-catenin. In terms of mucin phenotype, this thesis demonstrated evidence of post-tumorigenic mechanisms of MUC2 gene activation: GC developed on precursor lesions with intestinal metaplasia (IM) ”inherit” the MUC2-positive immunophenotype, but MUC2-positive tumors developed on MUC2-negative precursor lesions were also identified. In these cases, MUC2 positivity represents a post-tumorigenic acquision as a result of gene modulation by transcription factors or microRNAs. MUC5AC, a marker of gastric phenotype, together with VSIG1, can be used in metastases of unknown origin as evidence of gastric origin of the tumor.

A rare variant of squamous cell carcinoma (SCC) of the gastro-esophageal junction (GEJ), SCC with signet ring cells, was presented in Chapter 3. This subtype is extremely rare, with only 24 cases published in the literature. For a long time, the signet ring cell appearance was considered a feature of adenocarcinomas only, but recently it has been described in other tumors, such as lymphomas, sarcomas, mesotheliomas or SCC. The mechanism of occurence of this aspect in SCC is yet unknown. Electron microscopy studies have postulated the appearance of this variant as a result of dilation of endoplasmic reticulum cisternae.

Our study hypothesized for the first time the sequencing theory regarding TEM and stem cell feature aquisition as a mechanism involved in the occurence of this subtype. The acquisition of pluripotency, emphasized by SOX2 and/or CD44 positivity, is a mandatory step that precedes TEM initiation, but once the mesenchymal phenotype is completed, tumor cells lose stem cell features, which are no longer needed.