FONDAZIONE IRCCS ISTITUTO NAZIONALE DEI TUMORI

Sotto l'alto patrocinio
Presidenza del Consiglio dei Ministri

Loss of Succinate Dehydrogenase (SDHB) Expression in Midgut Carcinoids as Prognostic Factor: A New Marker of Personalized Cancer Medicine in Neuroendocrine Tumors?

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ABSTRACT

Background: Gene mutations of the succinate dehydrogenase (SDH) complex have recently been involved in the pathogenesis of cancer cells. These mutations are often associated with SDH subunits B loss of activity and overexpression of HIF-1α, which play a central role in angiogenesis and cell proliferation. The immunoistochemical (IHC) loss of SDHB expression has recently been reported to be a surrogate marker of malignancy in sporadic and familial pheocromocytomas and paragangliomas via activation of hypoxia signals.

Aims: To evaluate SDHB levels expression in advanced midgut carcinoids and investigate its potential role as prognostic marker.

Materials and methods: 31 advanced midgut carcinoids were treated in our Istitution, including 24 males and 7 females, aged 55.5 yrs on average (range 19-75). All patients were G1 tumors with stage IV for synchronous liver metastases. 25/31 patients underwent surgical primary resection. IHC evaluation of the SDHB and MIB1 expression were carried out in 19 primary tumors (T) and 19 liver metastases (M). In 11 patients SDHB and MIB1 were tested in both T and M. SDHB was assessed according to the staining intensity scored 1 (low) or 2 (high) on the basis of the internal control represented by normal intestinal cells.

Results: High (2+) positivity for SDHB, with clear cytoplasmatic mitochondrial reactivity, was found in 14/19 (77%) T, whilst loss of SDHB expression (1+) was detected in 17/19 (90%) M. The combined analysis (T+M) confirmed the loss of SDHB expression in 9/11 (82%) metastases compared to 2/11 (18%) primary tumors. These findings were inversely proportional to Mib-1 distribution that was 1,54% in metastatic sites and 0.7% in the primaries, respectively.

Conclusions: This preliminary analysis suggests a possible correlation among SDHB expression loss (score 1+), MIB1 increase and biological aggressiveness of advanced midgut carcinoids. A more extensive clinico-biological evaluation is warranted to clarify its relationship with survival and explore the role of SDHB as predictive marker to response of antiangiogenetic agents.

PATIENTS CHARACTERISTICS

mNET tumors from 31 patients (78% males and 22% females, median age 55.5 years, range = 19 to 75 years) were retrieved from the archives of the Pathology Department of the National Cancer Institute of Milan. These cases had been surgically treated from 1992 to 2007 at the Department of Surgery of the same Institutions. According to clinical and laboratory findings, two tumor groups were identified: the functional (FT) group was defined by the occurrence of a compatible clinical syndrome associated with the serum elevation and the immunohistochemical detection of the relevant hormones, and the non functional (NFT) group by the absence of both clinical symptoms and serum elevation of gastrointestinal hormones, regardless of the presence of immunostaining for any hormones. All cases were subjected to serum and immunohistochemical studies for CgA, synaptophisin, serotonin, and somatostatin receptor type 2A (SSTR2A). Twenty-two (70%) cases were up front treated with somatostatin analogues. Most of patients underwent surgical primary resection and all of them presented distant synchronous liver metastases. Three clinical subgroups of patients were thus considered according to the amount of liver involvement as assessed by surgical staging or CT scan: tumor load <25% (HI), between 25% and 50% (H2), and over 50% (H3).

Tumors specimens, immunohistochemical methods and scoring of data

The diagnosis of mNET was established by means of the last WHO classification criteria. All surgical samples (19 primary tumors, 19 metastases and in 11 combined primary and metastatic lesions) had been fixed in 10% buffered formaldehyde solution and embedded in paraffin. To minimize the intratumoral variability because of sampling process, the entire tumor was immunostained if the lesion was up to 2 cm in diameter or at least two representative tissue blocks were immunostained if the lesion was larger than 2 cm in diameter. Four µm-thick paraffin sections were reacted with monoclonal antibodies against CgA, synaptophysin, serotonin, Ki-67 antigen and SDHB and processed according to standard and previously refined immunohistochemical methods. Internal and external controls were used for all markers as appropriate.

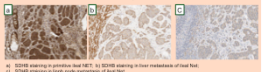
INTRODUCTION

Midgut neuroendocrine tumors (mNET) are the most common type of neuroendocrine neoplasms in the gastrointestinal tract, with a male prevalence and a median age at diagnosis of 66 years. They are mainly composed of enterochromaffin cells (EC) producing serotonin and substance P. Although in the past their diagnosis was based on the unique ability of tumor cells to incorporate biogenic amines, it is now accepted that immunohistochemistry for pan-endocrine markers, such as chromogranin A (CgA) and synaptophysin faithfully highlights these cells in human tissues. A distinctive feature of mNET, especially when involving the liver, is their capability of causing distinct clinical syndromes, which can be monitored over time by measuring in the bloodstream the relevant hormones. Although CgA is considered a general marker of neuroendocrine (NE) differentiation, a number of other markers are currently being investigated.

The succinate dehydrogenase (SDH) enzyme (also known as succinate ubiquinone oxydoreductase) is a highly conserved heterotetrameric protein, with SDHA and SDHB functioning as catalytic subunits, which protrudes into the mitochondrial matrix and is anchored to the inner membrane by means of SDHC and SDHD subunits, the latter also providing the binding site for ubiquinone. All these subunits are encoded by nuclear genes and then imported into the mitochondria, where they are modified, folded and assembled. Germ-line mutations in SDHD, -B and –C subunits have been observed in patients with hereditary paragangliomas and pheochromocytoma (HPGL/PCC) and rare somatic mutations have been detected in the corresponding spontaneous lesions. The genetic alterations in the SDH genes predisposing to the syndrome are germline heterozygous mutations, which cause inactivation of the protein function.

The prevalence of SDHB has not been thus far well documented in gastroenteropancreatic NE tumors, whereas many markers have been devised over time for highlighting NE differentiation, either cytosolic (Synaptophysin) or secretory granules (Chromogranins A-B). Moreover, the need to identify markers suitable for distinguishing subtypes of NE tumors with different biologic aggressiveness, especially in metastatic sites, is clinically warranted. In the current study, the status of SDHB immunoreactivity in a group of 31 mNET and corresponding metastases was explored in its diagnostic and prognostic implications.

FIGURE 1: SDHB Immunoreactivity:



a) SDHB staining in primitive ileal NET; b) SDHB staining in liver metastasis of ileal Net;
c) SDHB staining in lymph node metastasis of ileal Net

CONCLUSIONS

In this study, a number of these tumors show a Ki-67 proliferation index as low as 2%, and even within this subgroup of tumors, different degrees of aggressive behavior have been observed when SDHB expression was evaluated. Thus, it might be speculated that given the SDHB expression reported like SDHB score (intensity x x extension) could be one of the factors influencing tumor behavior by further decreasing the biological aggressiveness and contributing to this variable response. One of the most interesting findings in the present study was the marked heterogeneity of SDHB expression in the primary sites compared to metastatic ones. However, it has become increasingly employed in patients with documented progression of metastatic disease, even without symptoms related to hormonal hypersecretion, in the attempt to control tumor growth.

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