Correlation between IHH molecular pathway activation and tumor location, sex, age, distant metastases, in patients with diagnosed colorectal adenocarcinoma. Experimental study

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Abstract

Introduction: The Hedgehog (Hh) molecular pathway is especially important during embryonic development. Later in life it can affect the process of carcinogenesis. Specifically, in colon cancer, two ways of influencing the pathway are proposed, either with a paracrine role, where it creates a microenvironment conducive to carcinogenesis, or with its autocrine activation, promoting metastasis.

Methods: In tissue of 53 patients, the expression of the IHH index was estimated by immunohistochemistry and correlated with age, sex, tumor location and the presence of distant metastases.

Purpose: The aim of this study is to highlight the importance of the IHH index in the course of the disease either as a predictor or as a future therapeutic goal.

Results: The expression of the HH surface marker does not appear to be affected by the location of the tumor in the large intestine, similarly sex and age are independent elements, which are not related to the most common or not occurrence, of the above group of cancer stem cells. Essentially, however, the occurrence of distant metastases is statistically significant with \( p = 0.004 \), in patients with a higher incidence of HHH (+) cells, which makes their presence associated with a more aggressive form of the disease.

Discussion: It is clear that IHH (+) stem cells have a negative prognosis for patients with colorectal adenocarcinoma. They are associated with a more aggressive form, as the occurrence of metastases is more common in a statistically significant percentage compared to IHH (-) patients. This means that therapeutic targeting of these cells is a need for more desirable therapeutic results.

Introduction

The Hedgehog (Hh) molecular pathway is especially important during embryonic development. Later in life it can favor the process of carcinogenesis (Figure 1). Most basal cell carcinomas are characterized by mutations that lead to autonomic pathway activation \[1\]. Specifically, in colon cancer, 2 ways of influencing the pathway are proposed, either with a paracrine role, where it creates a microenvironment conducive to carcinogenesis, or with its autocrine activation, promoting metastasis \[2\].

Differentiated enterocytes secrete Indian Hedgehog (Ihh) ligase, which binds to the inhibitory receptor Patched 1 (Ptch1), leading to a decrease in the activity of the Smoothened receptor (Smo), resulting in the initiation of a signal sequence that activates the proteases associated with oncogene (Gli). The expression of these proteins is the most reliable indicator of molecular pathway activity \[3\].

Smo antagonists have recently been used in the treatment of basal cell carcinomas \[4\]. In trials also of patients with pancreatic and colon cancer to date, the results have been lower than expected \[5,6\] (Figure 1).

Study \[8\] investigated Hh levels in patients diagnosed with colon cancer. It was therefore found that the presence of Hh ligases was not proportional to the levels of Gli proteins. In several cases with KRAS mutation, there has been an increase in Gli1 \[9\]. However, no association was found between KRAS mutants and Gli1 mRNA. However, there was a weak correlation between APC mutation and low Gli1 levels. There is a different effect on the disease from the ligases in the layer with those directly related to the tumor cells.

Gorlin syndrome affects the development of basal cell carcinomas in various parts of the body. In patients with this syndrome, increased expression of Hh target genes was found without detecting mutations. In fact, the increased activity of the path concerned either the volume

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However, the IHH pathway also plays a role in the metastatic potential of cancer cells. In fact, the use of its inhibitors in pancreatic cancer appears to reduce the likelihood of metastasis [24,25]. In metastatic disease the IHH pathway is active not only in the tumor itself but also in the matrix microenvironment. The pathogenic factors that are mainly responsible for metastasis are not yet known, although there are several indications so far, eg snail, TGFβ, wnt, HGF [24,26,27].

**Methods**

The expression of the IHI index in archival material from the Laiko Hospital of Athens in 53 patients with diagnosed colorectal adenocarcinoma was studied by immunohistochemistry. This was followed by correlation with the age and sex of the patients, as well as with the location of the primary tumor in the large intestine and the presence or absence of distant metastases.

**Statistical analysis**

The Mann-Whitney U statistical test checks the equality of two means between independent samples. As a non-parametric test, it checks the correlation in populations in which normalcy cannot be ensured. The location of the cancer was defined as an independent variable. As the number of cancer cases located in the cation of the colon was small, two groups were formed, the first with the location of cancer in the orthosigmoid and the second with the location of the colon (anion or cation). The expression of the ihh index was the dependent variable in each case. 4 expression levels of each indicator were generated, depending on the signal strength of the cancer cells. The Spearmann Rho test was used to correlate age and IHH expression. Fisher’s exact test is used to look for correlations between categorical variables when the number of observations is small (<1000). It was used to find the statistical significance between the expression of the IHH index and the presence of distant metastases.

**Results**

The location of the primary tumor is not related to the increased or not, expression of the marker, therefore any differences between in particular anion and cation colon (Table 1), due to embryogenesis do not affect the presence of these cancer stem cells. Age also does not show a statistically significant correlation, which means that younger ages are not characterized by higher rates of IHH (+) cells and similarly there does not seem to be a decrease over time (Figure 2). Next, sex is an independent factor that does not affect cancer stem cells. Finally, the expression of the index is statistically significantly related to the occurrence of distant metastases with p = 0.004. This indicates the most aggressive form of the disease in patients with IHH (+) stem cells (Figure 3).

**Table 1. Frequencies of IHH score in the two major categories of colon**

| IHH SCORE | ORTHOSIGMOID | COLON |
|-----------|--------------|-------|
| 0         | 8            | 14    |
| 1         | 2            | 0     |
| 2         | 2            | 2     |
| 3         | 5            | 8     |
| 4         | 4            | 5     |
| 5         | 0            | 3     |
the disruption of communication with stromal cells. Inflammation of the large intestine promotes the development of sporadic cancer, so in studies in immunocompromised mice, the incidence of sporadic bowel disease has not been shown to protect against cancer.

The way the cancer pathway is activated, the differentiation of the relationship between cancer cells and the matrix, are the main points of the studies and the results are expected to help the medical community in the development of factors with good therapeutic results.

The results of our study are in line with the scientific knowledge so far, since the increased expression of the index is associated with the occurrence of metastases, making it necessary to find a therapeutic target.

Conflicts of interest

None.

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