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THE CELLULAR IMMUNE RESPONSE -CYTOTOXIC TYPE EXPRESSION IN THE TUMOR MICROENVIRONMENT OF SQUAMOUS CELL CARCINOMA

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Abstract

The malignant melanoma and squamous cell carcinoma are the two most common skin cancers. In the last years the higher number of cases are associated with the prelong time exposure to the UV radiations.

The clasic theory of the immune response states that a stronger response is corelated with a better prognosis for the cancer patientes. Taking as starting point this theory , our study goal is to analyze the cytotoxic-type immune respons in the peritumoral and intratumoral areas. The immunohistochemistry technique had been use to label CD8 lymphocytes. The statistic analyze was performed by hot-spot approche.

Our results reveals that the number of T-cell CD8+ in the peritumoral area is 39780 and it is 12188 in the intratumoral zone wich means that the most immportant role of the cyotoxic-type immune respons is in the peritumoral zone.

Key words: squamous cell carcinoma, skin, immune response, CD8+

INTRODUCTION

One of the most frecvent skin cancer type is squamous cell carcinoma. Inthe last years had been notice an increase of the number of skin caancer.

The clasic theory of the immune response states that a stronger response is corelated with a better prognosis for the cancer patientes. The last studies show that the tumoral immune response si more complex. This multilevel mechanism can play the protection role against cancer but some parts of the immune response may play the protumoral role.

Many authors confirmme that the skin is the most important rezervoir for T-cells. One of the explication could be the barrier role of the skin. (Boyman et al, 2007; Clark, 2010). Basically the immune respons presents two major components: celular immune response and humoral immune response. The first type of the immune response is based on the T-

cell function. The antibodies production by activated B-cells belongs to the humoral immune response.

Normally the T cytotoxic cells response (CD8+) and the T helper cells activity (CD4+) are the major two parts of the cellular immune response. Each lymphocites population has the own role; CD8+ cells are acting directly against tagets cells meantime the CD4+ cells are healping other immune cells to onset diferent mechanisms of action.

Takeing as starting point the studies wich correlate the immune respons and the cancers our study analizye the CD8+ lymphocites in the tumoral microenviromennt.

MATERIAL AND METHOD

In our study we included 35 cases of squamous cell carcinoma, random chose, from the files of Patology Department of Municipal Hospital from Marghita town. The study is a retrospective one from 2016.

All the tissue samples are stain with hematoxilin-eosin and had been diagnosis by two skill pathologist according with WHO classification. An immunohistochemical analysis was performed on 4 μm-thick sections prepared from formalin-fixed paraffinembedded tissue by using an automated immunostainer (Bechmark XT, Ventana Medical Systems Inc., Tucson, AZ, USA). Immunohistochemical assays were performed on a Ventana Benchmark GX automated staining instrument according to the manufacturer's instructions. The CD8 reactivity sections were incubated with the primary monoclonal rabbit antibody, clone SP57 (ready-to-use Ventana), according to the manufacturer's instructions. (www. ventana.com). The tonsil had been use as a positive control, meantime by primary antibody omitted stain was the negative control.

The microscopical analyze was done by hot-spot technique.

The Leika 300DM microscope with HD video camera was use to capture tissue images and the softwer provided by Leika company had been use to analyze the cases.

RESULTS AND DISCUSSION

If we analyze the immune response in the two areas studied by us show a big differences. The tumoral microenviroment reveals that the exression of CD8+ cells in the intratumoral zone is very low compare with the same expression in the peritumoral zone. (12188 vs. 39780).

Inside the tumoral bad the limits range from 137 CD8+ cells to 732 CD8+ cells. The peritumoral zone show a range from 427 CD8+ cells to 2637 CD8+.

The mean value in the two areas studied in our study are 324 CD8+ lymphocites intratumoral and 1137 CD8+ lymphocites in peritumoral zone. (fig.1)

To be more accurate we compared our results with the normal level of CD8+ cells in the skin. The data was provide by an international study showing a mean value of 15.67 CD8+ lymphocites. (4)

Limfocite CD8+	Intratumoral	Peritumoral
Nr. total	12188	39780
Nr. mediu	348	1137
Nr. minim/maxim	137 / 732	427 / 2637
Lot martor. Nr mediu	15.67	15.67

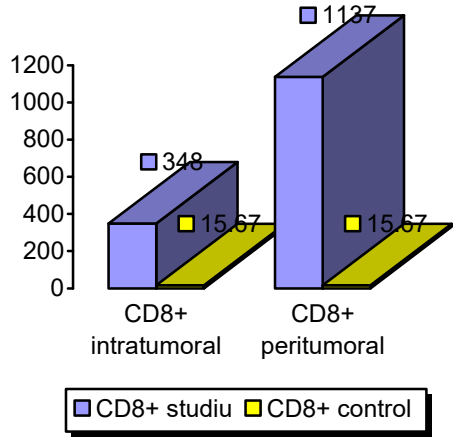

Fig.1 Analyze of the Tc intratumoral and peritumoral areas and the normal skin mean value

The ration of CD8+ cells between intratumoral areas and peritumoral areas is 0.3 with means that the cytotoxic immune response is more immportant in the peritumoral areas.

When the data are transforms in the percent we see that 30.60% of Tc lymphocites , from total number, is inside tumoral bad.(fig. 2)

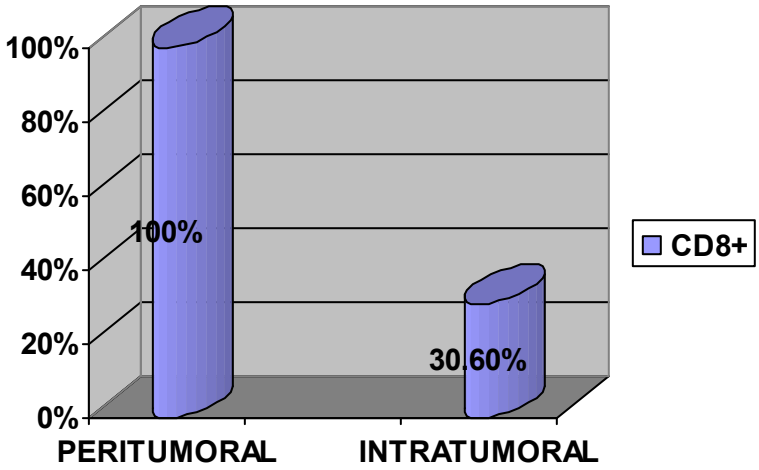

Fig. 2 nr. Percental analyze of the Tc intratumoral and peritumoral areas

The next image show the immunohistochemistry expression for CD8+ in our study. The CD8+ lymphocites are label by DAB chromogen in brown.

Inside tumoral bad can be seen few Tc lymphocites and in the edge of the squamous cell carcinoma can be noticed a dense Tc lymphocites infiltration. Between the two area included in our study is very sharpe. (fig3.)

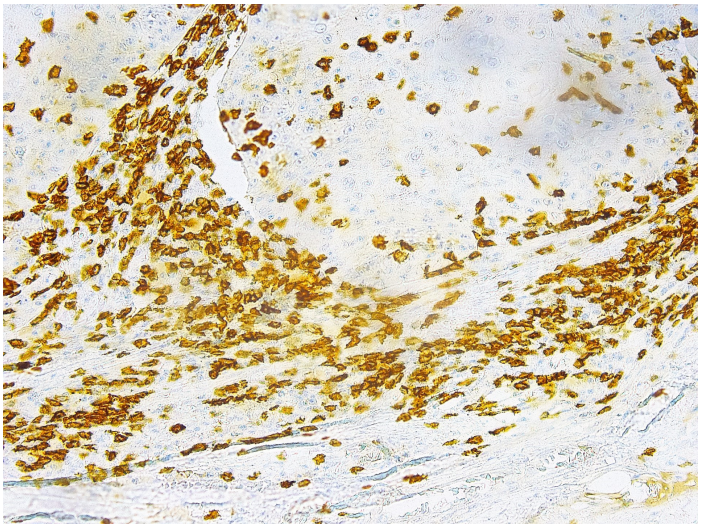

Fig. 3 T cytotoxic lymphocites (CD8+) expression

CONCLUSIONS

Numerous studies have been conducted to assess the role of tumor infiltrating lymphocytes (TILs) and their possible association with a better prognosis.

In a related study assessing immune response in cervical carcinomas states that an increasing number of TILs is associated to a decrease in survival rates for these patients (Ekaterina S., 2008).

In our opinion these results are not very conclusive given the fact that the aforementioned study does not evaluate lymphocytes taking in consideration their subpopulations.

The importance of T cells in the peritumoral areas is correlated to tumor progression if these lymphocyte populations decrease in number due to immunosuppressive therapy.

In our study we observed that the distribution of values TCD8 + lymphocytes (cytotoxic) is different between the two areas studied: the intratumoral and peritumoral areas. CD8 + lymphocytes distribution charts for intratumoral areas reveals a smaller variation relative to the median in comparison with the results obtained for the peritumoral area.

For the majority of the studied cases, there is a clear separation between the immune response trend in the two distinct areas, but there have been cases considered by us particular presenting either a small number of cytotoxic T lymphocytes (CD8 +) either in both areas or large numbers of cytotoxic T lymphocytes (CD8 +).

The ratio between the average number of cytotoxic T lymphocytes in normal skin and the number of lymphocytes in the two areas studied reveals a huge difference in favor for tumor immune response. The ratio for the intratumoral area was 22.2 while in peritumoral area the ratio was 72.55. Our measurements confirm the statistical value of the results obtained in our study.

Both cytotoxic T lymphocyte expression analysis of the two studied areas and expression analysis of tumor cytotoxic immune response compared with the presence of CD8 + lymphocytes in the skin reveals the importance of peritumoral cytotoxic cellular immune response as an antineoplastic factor.

In colorectal carcinomas, if an increased ratio of CD8 / Treg , the risk of death is reduced by 70%. One explanation could be the inclusion of Cyclophosfamide in the chemotherapy scheme for these patients. It turned out that Cyclophosfamide reduces the number of Treg cells favoring anti-tumor immune response (Frank, 2009).

It was also demonstrated that chemotherapy changes the blood's lymphocyte T count in accordingly to the different subpopulations

belonging to this family of immune cells. It is not yet clear what actually happens at the tissular level. Larger studies are needed in this regard (van Hall et al 2006).

The generally accepted theory is that the immune response mediated by lymphocytes CD4 + Th1 and CD8 + T is the protective antitumor factor and lymphocytes belonging to populations CD4 + Foxp3 + Treg favor the development and progression of tumors through the release of several cytokines involved in local immunosuppression.

The association between the increased number of lymphocytes TCD8 + and increased survival rate was also seen in other studied tumors: ovarian carcinoma, colorectal carcinoma, endometrial carcinoma, malignant melanoma (van Hall et al 2006; Petersen et al., 2006; Gao et al., 2007).

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