doi: 10.5216/rpt.v53i1.77403

ORIGINAL ARTICLE

EVALUATION OF INTERLEUKIN-23 EXPRESSION IN CLASSIC KAPOSI'S SARCOMA AND AIDS-ASSOCIATED

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ABSTRACT

Kaposi's sarcoma (KS) is a low-grade vascular tumor that presents most frequently with skin lesions. Multiple factors are related to KS pathogenesis. The interleukin-23 (IL-23) is an immunomodulatory cytokine with pro-carcinogenic role. The aim of this study was to evaluate the detection of IL-23 in cutaneous lesions of patients with KS classic and KS-AIDS. This was a cross-sectional study conducted at the public Hospital in Natal, Rio Grande do Norte, Brazil. This study included 13 biopsies of patients which presented KS with histopathological confirmation. For detection of IL-23, the immunohistochemical analysis was made with monoclonal antibody anti-IL-23. The immunostaining for IL-23 in biopsies was positive in 61.5% (8/13) of cases. Of seven lesions classified as patch-stage, three were positive for IL-23 (42.8%); two of three samples classified as plaque-stage were positive for IL-23 (66.7%); all the nodular-stage lesions were positive for IL-23. The positivity found in KS-AIDS lesions was 66.7% (6/9) and KS classic 50% (2/4). Our data supports the concept that IL-23 might be a pro-carcinogenic mediator, detected in KS lesions, probably favoring the progression of the tumor.

KEY WORDS: Sarcoma; Kaposi; interleukin-23; tumor microenvironment.

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Received for publication: 9/10/2023. Reviewed: 18/12/2023. Accepted: 11/1/2024.

INTRODUCTION

Kaposi's sarcoma (KS) is a vascular tumor associated with infection of human herpesvirus 8 (HHV-8) and it has four clinical and epidemiological forms (KS classic, KS endemic, KS post-transplant and KS-AIDS) with very similar histopathological features, as proliferation of spindle cells, inflammation and neo-angiogenesis (Ensoli et al., 2001; Gessain & Duprez, 2005).

HHV-8 contains a double stranded DNA, and it may remain latent in the host as other herpesviruses. This virus is associated with the development of hematological malignancies as well as the Epstein-Barr virus (EBV) (Cesarman et al., 2022). Transmission of this virus occurs mainly by saliva. Seven different subtypes of HHV-8 have been reported and its distribution varies geographically throughout the world (Malonga et al., 2021).

Patch-stage, the KS initial phase, is characterized by a subtle angiogenic process, with the presence of little irregular newly formed vascular spaces (Grayson & Pantanowitz, 2008). In this stage of the lesion, sparse chronic inflammatory cells are commonly found, together with macrophages and extravasated erythrocytes (Radu & Pantanowitz, 2013). The plaque-stage is the next stage, characterized by an increase in the proliferation of spindle cells and new vessels, occupying a large part of the dermis (Radu & Pantanowitz, 2013). There is scarcity of mitotic figures, and it is possible to find extra and intracellular hyaline globules. In the late-stage lesions called nodular-stage, it may be possible to find in the dermis an exacerbated proliferation of spindle cells arranged in fascicles, almost always monomorphic (Grayson & Pantanowitz, 2008).

Depending on the stage in which the KS lesions are, a variable quantity of cells may be found, as endothelial cells, spindle cells and cells from the inflammatory infiltrate. The spindle and inflammatory cells, in association or individually, are responsible for the production of several inflammatory cytokines and chemokines (Gessain & Duprez, 2005).

Interleukin-23 (IL-23) is an immunomodulatory cytokine involved in the regulation of inflammatory response in the tumor microenvironment and with potential pro-carcinogenic effect (Kortylewski et al., 2009; Yannam et al., 2012). Thus, the aim of this work was to evaluate the detection of IL-23 in cutaneous lesions of patients with KS-AIDS and KS classic.

MATERIAL AND METHODS

This study included 13 patients who presented skin lesions characteristics of KS, with confirmed histopathology, being 12 men and one woman, with average in age of 35 years old. The skin biopsies were obtained from the archives of the Department of Pathology at the Federal University of

Rio Grande do Norte. Within our cohort, nine patients with KS-AIDS and four patients with KS classic were enrolled. The local ethics committee approved this study (CAAE - 02097912.1.0000.5292).

The immunohistochemical assays for the detection of the IL-23 were carried out with tissues fixed in formalin and paraffin embedded. Antigenic recovery was obtained with citrate 10mM, pH 6.0, in a pressure cooker for 10 minutes. The activity of the endogenous peroxidase was blocked with addition of 3% hydrogen peroxide in methanol (v/v) for 20 minutes. The slides were blocked with 1% fat-free milk for 30 minutes. After that, the slides were incubated, adding anti-IL-23 P19 monoclonal antibody (dilution 1:50) (ab41545 clone; Abcam®, Massachusetts, EUA) for 3 hours, washed, and incubated with MACH 4 universal HRP Polymer detection® (Biocare Medical, EUA) for 40 minutes. The slides were developed with diaminobenzidine (DAB®) (Dako) and counter-stained with Harris's hematoxylin. The reaction was considered positive for IL-23 when spindle cells showed brown cytoplasmic staining and negative when cells showed blue staining.

The slides of KS lesions were analyzed by pathologists and spindle cells with cytoplasm brown color were considered positive. The pathologists assessed the magnitude of IL-23 expression in a semi-quantitative way subjectively attributing scores in a scale ranging 0 to 3 (0=none, 1=weak, 2=moderate, 3=strong). To validate the anti-IL-23 mAb and the immunohistochemical method, we systematically analyzed a paraffin-embedded section of kidney tissue (positive control). Basal IL-23 expression was evaluated in normal skin biopsies obtained at plastic surgery. A negative control was performed by omitting the primary antibody.

RESULTS

The KS lesions were classified histologically in three groups: patch-stage KS (54%), plaque-stage KS (23%) and nodular-stage KS (23%).

The immunostaining for IL-23 (Figure) was positive in 61.5% (8/13) of the cases (Kappa value 1,0; $p<0.001$). Three out of seven samples classified as patch-stage were positive for IL-23 (42.8%), two of the three samples in plaque-stage were positive for IL-23 (66.7%) and all three samples in nodular-stage lesions were positive (100%).

Negative staining was noted in five tumors (38.4%) (four cases of patch-stage and one plaque-stage). Of the KS positive lesions, three were weakly positive (one patch-stage and two plaque-stage), four moderately positive (two patch-stage and two nodular-stage) and one strongly positive, classified in nodular-stage.

In relation to the KS type, the positivity found in KS-AIDS lesions was 66.7% (6/9) and KS classic 50% (2/4).

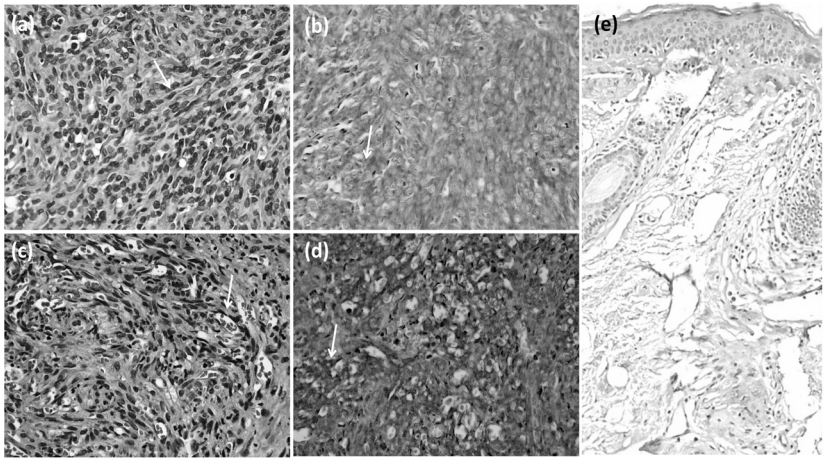

Figure. IL-23 detection in skin specimen from patient with Kaposi's sarcoma. Skin biopsy was analyzed by haematoxylin–eosin (H&E stain) and immunohistochemistry using anti-IL-23 P19 antibody. (a) Lesion constituted by neoplastic proliferation of elongated cells with eosinophilic cytoplasm and oval or rounded nucleus (white arrow) (H&E stain). (b) Positive immunostaining for IL-23 of spindle cells (brown staining - white arrow) in nodular-stage KS. (c) Neoplastic proliferation of elongated cells with formation of slits containing erythrocytes in its interior (white arrow) (H&E stain). (d) Positive immunostaining for IL-23 of spindle cells (brown staining - white arrow) in KS. Original magnification: ×400 (e) Control skin (normal skin) negative for IL-23 (blue staining). Original magnification: ×100.

DISCUSSION

Several neoplasias have their origin linked to infectious agents, which generate chronic inflammatory symptoms (Yannam et al., 2012). In the case of KS, several inflammatory cytokines like IFN-g, TNF-α, IL-1β, IL-2 and IL-6 accentuate even more these symptoms (Ensoli et al., 2001).

Santarelli et al. (2014) demonstrated that DC exposure to active or UV-inactivated KSHV resulted in STAT3 phosphorylation. This effect, partially dependent on KSHV-engagement of DC-SIGN, induced a high release of IL-10, IL-6 and IL-23, cytokines that in turn might maintain STAT3 in a phosphorylated state.

In chronic inflammation, the immunologic response cells act secreting different cytokines and growth factors which stimulate the angiogenesis, inducing the tumor growth. Inflammatory cells in the tumor microenvironment contribute actively to tumor cells proliferation and survival, which makes it even more favorable for malignant development (Yannam et al., 2012).

Based on our results, it was demonstrated for the first time that spindle cells are a potential source of IL-23 in KS lesions, suggesting that IL-23 might have a role in KS tumorigenesis. Studies suggest a pro-carcinogenic role of IL-23, being this cytokine over-expressed in animal and human tumors (Kortylewski et al., 2009; Yannam et al., 2012). Furthermore, it was observed that mice depleted of IL-23 cytokine and its receptor presented a reduction in the growth of transplanted tumors (Langowski et al., 2006). Studies show the relationship between IL-23 and the development of different neoplasia, like the colorectal carcinoma (Lan et al., 2011) and the squamous cell carcinoma (Fukuda et al., 2010).

These results suggest that positivity is higher in late-stage lesions, and we observed that all nodular KS lesions were positive for IL-23, showing from moderate to strong staining, probably favoring the progression of the disease. Studies show that there is an inversely proportional relationship between IL-23 and the presence of cytotoxic T lymphocytes, with a reduction of these cells in the tumor due to the presence of IL-23, showing association of this cytokine with neo angiogenesis (Langowski et al., 2006; Kortylewski et al., 2009).

Guedes et al. (2008) observed that the number of cytotoxic T lymphocytes in KS classic and KS-AIDS lesions, with or without the use of highly active antiretroviral therapy (HAART), is similar, as it was verified that these cells are in minor quantity in the cutaneous lesions of the nodular type when compared to the patch and plaque stages.

In conclusion, it is possible to note that as the KS cutaneous lesions become more severe, a major positivity for IL-23 was verified, suggesting a probable correlation between the severity of the lesion and the detection of this cytokine. Nevertheless, further studies are necessary to reinforce this possibility.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest to disclose.

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