Original Article

Differential Diagnosis of Gingival Hyperplasia Based on IFN-γ-Stimulated Gene Expression Using Oligonucleotide Microarrays

(epulus / gingival squamous cell carcinoma / oligonucleotide microarrays / IFN-γ / apoptosis / proliferation)

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Abstract. The aetiopathogenesis of epuli, a group of benign gingival hyperplasias, remains unclear. The purpose of the present study was to determine similarities and differences between benign (epulus) and malignant (cancer) gingival hyperplasias based on the evaluation of three signal pathways stimulated by the IFN-γ complex. Five gingival specimens sampled from giant cell epulus, fibrous epulus, central giant cell granuloma, high- and low-differentiated carcinoma were involved in the investigations. Based on literature data, a map was designed/developed, and selected genes of three signal pathways stimulated by the IFN-γ complex were marked on the map. Gene expression was compared on five oligonucleotide microarrays. In molecular analysis, giant cell epulus shows characteristics of a neoplastic lesion, while central giant cell granuloma constitutes a separate diagnostic entity different from epuli and carcinoma.

Introduction

The knowledge of the aetiopathogenesis of benign and malignant gingival hyperplasias is incomplete. Epuli (granulomas) are the most frequently observed gingival tumours. Literature on the subject presents considerable discrepancies regarding classification, aetiopathogenesis, and management of the lesions (Axhausen, 1940; Bernier and Cahn, 1954; Kumar et al., 1997). Immunohistochemical studies have shed some light on the role of apoptosis and proliferation in the development of this entity (Pammer et al., 1998; Souza et al., 2000). However, the literature does not present any molecular profile of the tumours. The actions of pleiotropic interferon (IFN)-γ and its receptor complex are still unclear; whether it might emerge as a diagnostic-therapeutic marker remains to be elucidated. It should be emphasized that the active IFN-γ receptor (IFN-γR) is a dimer consisting of two subunits, capable of stimulating signal pathways causing cell responses in the form of apoptosis or proliferation. We have not found any literature report on IFN-γ and IFN-γR1, IFN-γR2 analysis in epuli.

The purpose of the present study was then to determine similarities and differences between benign (epulus) and malignant (cancer) gingival hyperplasias based on the evaluation of three signal pathways stimulated by the IFN-γ complex.

Material and Methods

Five patients were enrolled in the pilot study. Based on macro- and microscopic analyses the most homogenous gingival specimens were selected, sampled from giant cell epulus, fibrous epulus with an inflammatory component, high- (GI) and low-differentiated (GIII) carcinoma; an additional specimen taken from a patient with central giant cell granuloma was also included in the investigations.

The investigations were carried out on TRIzol-extracted RNA, which was then purified and digested with DNase I. About 8 μg of total RNA were used in the synthesis of double-stranded cDNA. The next stage of the process was synthesis of biotin-labelled cRNA, which was subsequently fragmented and hybridized onto Test 3 and HG-U133A (Affymetrix, Santa Clara CA) microarrays. Following that, streptavidine-phycoerythrine complex labelling was used; fluorescence intensity was measured with GeneArray Scanner G2500A.

The quantity and quality of total RNA, cDNA and cRNA was assessed with spectrophotometry, and in 1.2% agarose gel electrophoresis. The analysis of results was carried out using MicroArray Suite 5.0, Data Mining Tool (Affymetrix), Cluster 3.0, RMA Express.

Based on literature data, the map itself was designed/developed (Fig. 1) with representations of IFN-γ/IFNγ-R1/IFNγ-R2 stimulation of genes participating in the following processes:

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Abbreviations: IFN – interferon, IFN-γR – IFN-γ receptor.

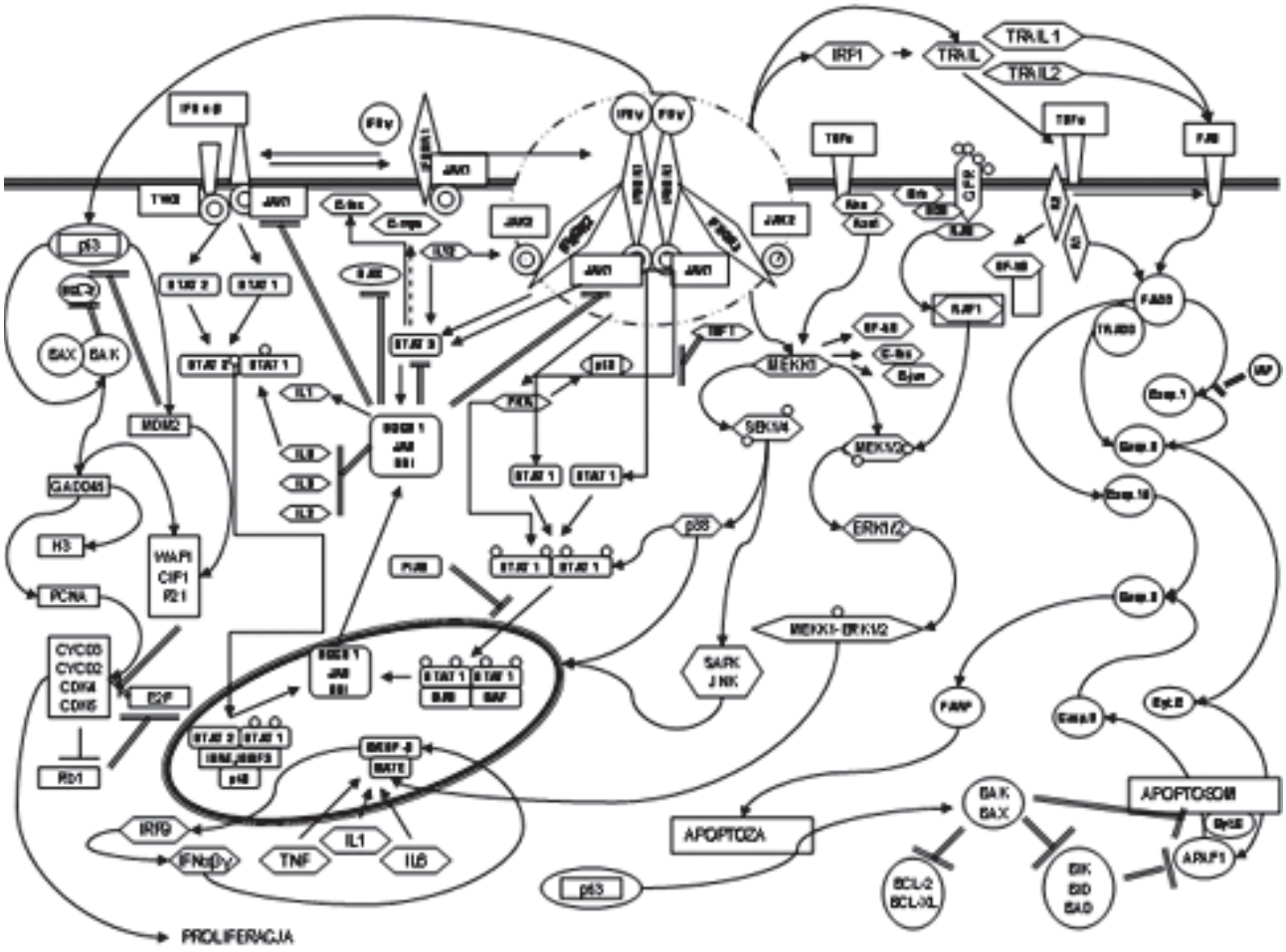

Fig. 1. The map with representations of IFNγ/IFNγR1/IFNγR2 stimulation of genes participating in the following processes

apoptosis: *IRF-1, TRAIL, TRAIL-1, TRAIL-2, TNF-α TNF-R1, TNF-R2, FAS, FADD, TRADD*, caspases 1, 3, 8, 9,10, cytochrome c, *bcl-2, bax, bik, APAF-1, PARP, p53* (Takeda et al., 2001a, b; Ahn et al., 2002; Kamei et al., 2003; Ahang et al.; 2004 Inaba et al., 2004),

proliferation: p53*, bcl-2, bax, MDM-2, GADD-45*, H3 histone, *PCNA, WAF-1/CIF-1/p21, CYCD-2, CYCD-3, E2F, Rb-1* (Bianchi et al., 1989; Fornace et al., 1988; Hauck and Harsdorf, 2005; Kovalsky et al., 2001),

inflammation: *TNF-α, Rho, Rac-1, GFR, Grb, SOS, Ras, RAF-1, MEKK-1, MEK-1/2, SEK-1/4, ERK-1/2, MEKK-1/ERK-1/2, SAPK/JNK, p38* (Baud et al., 1999; Stancato et al., 1998; Xia et al., 2000).

Using fluorescence intensity, the role of genes of similar and different expression profiles was investigated in selected gingival hyperplasias. Fluorescence intensity served as a criterion for identifying gene expression determined by the technique of oligonucleotide microarrays.

Results

The fibrous epulus sample showed high fluorescence of the IFN-γ complex; expression patterns of receptor subunits were comparable. IFN-γ/JAK/STAT pathway genes as well as the pathway inhibitors demonstrated the same high-level stimulation as IFN-γ receptor subunits. The inflammation pathway, induced by highly active *Rho* and *Rac-1*, *TNF* genes and IFN-γ complex, showed high level expression of *MEKK-1, SEK-1/4* and *SAPK*, and distinctly weaker *MEK-1/2, ERK-1/2, MEKK-1/ERK-1/2* expression profiles. Caspase 1, *PARP*, and cytochrome c showed the highest expression patterns in apoptosis. The expression of the process triggers, i.e., *IRF-1, TRAIL* and *fas*, was also quite pronounced. In proliferation, high fluorescence intensity was observed for *cdk-4* and *cdk-6*, while it was moderate for *PCNA* and *p53*. The weakest expression patterns were detected for *MDM-2*, *GADD-45*, H3 histone, and *p21*.

Giant cell epulus sample – fluorescence intensity of IFN-γ/JAK/STAT pathway genes was less pronounced in comparison to fibrous epulus while IFN-γ receptor subunits showed the same expression level. In both epulus types, the activity of JAK/STAT pathway inhibitors (PIAS, SOCS) was equal to the activity of the process genes. Giant cell epulus was characterized by high-level expression of several inflammation pathway genes,

mainly *ERK, MEKK-1/ERK-1/2. MEKK-1* and *SEK-1/4* expression was less pronounced. Fluorescence intensity was suggestive of greater activity of *Rho* and *Rac-1* in the induction of the inflammation pathway. *bax*, *bak*, caspase 8, and apoptosome-*APAF-1* component exhibited higher level expression when compared to that of fibrous epulus; caspase 1 and cytochrome c expression profiles remained unchanged. *p53* demonstrated similar expression levels in all analyses. *GADD-45* expression was more pronounced in giant cell than in fibrous epulus.

Central giant cell granuloma – IFN-γ receptor subunit IFN-γR2 was inactive while IFN-γR1 exhibited only weak expression.

IFN-γ/JAK/STAT pathway genes demonstrated the lowest expression level of all cases; similarly as *Rho* and *Rac*. TNF expression was lower than in fibrous epulus and higher in comparison with giant cell epulus. The inflammatory process was characterized by *p38, MEKK-1/2, ERK-1/2, MEKK1-ERK-1/2* expression. Among IFNγ-induced apoptosis genes, high-level fluorescence was observed for *bak, bax, bik, bid*, and *APAF-1*. In the process of proliferation, the highest expression levels were seen in the case of *Rb-1, cdk-4*, and H3 histone.

In gingival cancer (high- and low-differentiated), IFN-γR2 expression was more pronounced than that of IFN-γR1. Low-differentiated carcinoma exhibited *STAT-1* and *STAT-3* overexpression while the JAK/STAT inhibitor (PIAS) only demonstrated moderate stimulation.

TNF was inactive in both types of cancerous cells. High-differentiated carcinoma exhibited higher fluorescence levels of inflammatory *MEKK-1* and *SEK-1/4,* whereas *ERK-1/2, MEKK-1/ERK-1/2*, and *SAPK* were activated in low-differentiated carcinoma.

Of all genes analysed for gingival malignancies, *Rac-1* demonstrated the highest expression level. The expression profiles of apoptosis genes were comparable for both types of cancer.

Higher fluorescence intensity of cytochrome c was observed in gingival malignancies than in benign lesions.

Discussion

Classification, aetiopathgenesis, and management of epuli have long been subject of controversies. Clinically, the hyperplasias are most frequently classified as inflammatory, fibrous, and giant cell epulus; all have been considered to be of inflammatory rather than of neoplastic origin (Anderson and Jones, 1970; Eversole and Rovin, 1972, 1973; Demetrion, 1973). An analysis of both benign and malignant gingival hyperplasias has provided new insight into their nature. Central giant cell granuloma was included in the control; despite different location, it reminds histologically of giant cell epulus. According to Souza et al., (2000), the former is more aggressive, and yet its proliferation pattern is weaker (Souza et al., 2000). The tumour was first described by Jaffe in 1953. Central giant cell granuloma most frequently develops following trauma in young people; it arises in the bones of the facial skeleton, more often in the jaw than in mandible. Jaffe (1953) described slow progress; other authors mention aggressive growth and recurrence (Stolovitzky et al., 1994). Molecular analysis performed in our study has revealed considerable differences between central giant cell granuloma and epulus/cancer; in fact, the lesion seems to constitute a separate disease entity.

The characteristics of giant cell epulus are more similar to those of high-differentiated squamous cell gingival carcinoma than it is in the case of other epulus types. Considering the activity of apoptosis and proliferation genes, it can be classified as a type of neoplastic tumour, and thus possibly also as a separate diagnostic entity.

However, regarding the small number of cases investigated using the technique of oligonucleotide microarrays (high cost), classification of the above-mentioned lesions as separate entities is by no means definite.

However, the analyses of apoptosis and proliferation pathways have disclosed considerable similarities between giant cell epulus and GI gingival carcinoma, whereas central giant cell granuloma showed completely different characteristics.

Conclusions

In molecular analysis, giant cell epulus shows characteristics of a neoplastic lesion, while central giant cell granuloma constitutes a separate diagnostic entity different from epuli and carcinoma.

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