**Online supplement**

**Presence of tertiary lymphoid organ in nasal inverted papilloma is correlated with eosinophil infiltration and local immunoglobulin production**

**MATERIALS AND METHODS**

**Immunohistochemical and immunofluorescent experiments**

Immunohistochemical experiment: The tissue sections were first performed antigen retrieval by pretreating with retrieval buffer (Dako, Glostrup, Denmark), then stained with respective primary anti-human antibodies by overnight incubation at 4℃, and incubated with DAKO EnVision+ System-HRP at room temperature for 30 minutes followed by staining with diaminobenzidine (DAB) for color development.

Immunofluorescent experiment: Tissue sections were performed antigen retrieval and staining of primary antibodies, and goat anti-mouse Alexa Fluor 488 and goat anti-rabbit Alexa Fluor 594 conjugated secondary antibodies (Thermo Fisher Scientific, Waltham, MA) followed by counterstained with 4’,6’- diamidino-2-phenylindole (DAPI) for nucleic acid staining.

Evaluation methods: the evaluation of the eosinophils, neutrophils, Tfh cells, and immunoglobulin producing cells were performed in three randomly selected regions at a 400x magnification and the positive cell number was manually counted in the captured pictures; the mean value was calculated by the total positive cell number for each sample. Moreover, the above positive cells were not counted if they were infiltrated in epithelium area or inside the small blood vessels in the laminar propria. The tissue sections were coded by the number and two researchers (Q.B & X.X.G in the author list) independently assessed all cases. Where discrepancies in evaluation arose, a blind third researcher (C.C in the author list) evaluated the section in question independently and then both researchers to resolve the discrepancy.

**RNA extraction and quantitative RT-PCR**

The total RNA was extracted from the tissues using the Trizol reagent (Thermo Fisher Scientific) according to the manufacturer’s instructions. Total RNA was reverse-transcribed to complementary DNA by using a Prime Script RT Kit (Takara Bio, Tokyo, Japan). Selected genes were analyzed by real-time quantitative PCR by using SYBR Premix Ex Taq (Takara Bio), and the experiment was performed on ABI 7300 real-time PCR system (Thermo Fisher Scientific).

**Tissue homogenates and Luminex assay**

NIP tissue specimens were homogenized for 5 minutes with PBS containing a protease inhibitor cocktail by using a Bullet Blender Blue homogenizer (Next Advance); and then the homogenate was centrifuged and the supernatants were collected for cytokine analysis. Cytokine levels of IL-1β, IL-2, IL-6, TNF-α, IL-12p70, IL-18, IFN-γ, IL-4, IL-5, IL-9, IL-13, IL-17, IL-21, IL-22, IL-23, and GM-CSF in tissue homogenates were measured by customized ProcartaPlex assay (Thermo Fisher Scientific) following manufacturer’s instructions. The experiment was performed by using a Luminex Magpix system (Luminex, Austin, Tex). The raw data was measured as mean fluorescence intensity (MFI) and the concentration of each analyte for each sample was calculated using a 5-parameter logistic fit curve generated for each analyte from the standards.

**Table E1** Characteristics of NIP patients

|  |  |  |
| --- | --- | --- |
| Clinical Parameters | Patient number (n=84) | *%* |
| Age, years, median (1st & 3rd interquartile) | 51 (43, 62) | N/A |
| Gender |  |  |
| * Male
 | 69 | 82% |
| * Female
 | 15 | 18% |
| Smoking\* |  |  |
| * Smoker
 | 29 | 35% |
| * Non-smoker
 | 53 | 65% |
| Nasal polyp (NP)# |  |  |
| * With NP
 | 28 | 34% |
| * Without NP
 | 54 | 66% |
| Recurrence |  |  |
| * First diagnosis of NIP
 | 45 | 54% |
| * Recurrent NIP
 | 39 | 46% |
| Krouse Staging system |  |  |
| * Stage I
 | 5 | 6% |
| * Stage II
 | 19 | 23% |
| * Stage III
 | 60 | 71% |
| * Stage IV
 | 0 | 0 |

\*2 patients were without information on smoking.

#2 patients were without information on nasal polyps.

**Table E2** Antibody information

|  |  |  |
| --- | --- | --- |
| **Target antigen** | **Species** | **Clone** |
| * CD3
 | Mouse | PS1 |
| * CD4
 | Mouse | 4B12 |
| * CD8
 | Mouse | C8/1448 |
| * CD20
 | Mouse | EP459Y |
| * CD21
 | Rabbit | EP3093 |
| * CD56
 | Rabbit | EP2567Y |
| * CD68
 | Mouse | C68/684 |
| * CD138
 | Mouse | B-A38 |
| * CXCR5
 | Rabbit | D6L3C |
| * IgA
 | Rabbit | EPR5367-76 |
| * IgM
 | Rabbit | EPR5539-65-4 |
| * IgG
 | Rabbit | IG507R |
| * IgE
 | Rabbit | RM122 |

**Table E3** Primers used for quantitative RT-PCR analysis

|  |  |
| --- | --- |
| **Primer** | **Sequence** |
| CXCL12 | (F) 5’-ATTCTCAACACTCCAAACTGTGC-3’ |
| (R) 5’-CTTCAGCCGGGCTACAATCTG-3’ |
| CXCL13 | (F) 5’-GGACTCAGAGCTCAAGTCTGAACTC-3’ |
| (R) 5’-CAGCAGCATGAGAAGCAGAGA-3’ |
| CCL19 | (F) 5’-TGTCTGTGACCCAGAAACCCA-3’ |
| (R) 5’-TGAACACTACAGCAGGCACCC-3’ |
| CCL20 | (F) 5’-CTGTACCAAGAGTTTGCTCC-3’ |
| (R) 5’-GCACAATATATTTCACCCAAG-3’ |
| CD21L | (F) 5’-TGGAACCTGGGATAAACCTGC-3’ |
| (R) 5’-GACTTGTTTCCGTTCATGGAGA-3’ |
| LTα | (F) 5’-ATGACACCACCTGAACGTCTC-3’ |
| (R) 5’-CTCTCCAGAGCAGTGAGTTCT-3’ |
| LTβ | (F) 5’-AGGGTGTACGTCAACATCAGTCA-3’ |
| (R) 5’-TATTCACGCACTCGCACCA-3’ |
| GAPDH | (F) 5’- GAGTCAACGGATTTGGTCGT-3’ |
| (R) 5’-TTGATTTTGGAGGGATCTCG-3’ |

**Figure E1** Correlation analysis of Immunoglobulin producing plasma B cells (CD138+IgM+, CD138+IgA+, CD138+IgG+, CD138IgE+), Tfh cells and eosinophils in NIP tissues (n=40). *r* value, correlation coefficient.