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LETTER TO EDITOR

A linking bridge between histopathological analysis and molecular assay in microbiology

Yoichiro OKUBO *MD, PhD*

Department of Pathology, Kanagawa Cancer Center, 2-3-2, Nakao, Asahi-Ku, Yokohama, Kanagawa, 241-8515, Japan

Dear Editor,

Cryptococcus gattii R265, which was responsible for the cryptococcosis outbreak on Vancouver Island, can cause life-threatening infectious diseases.[1] However, putative mechanisms of its high virulence remain unclear. To elucidate factors affecting the high virulence of this yeast, we conducted histopathological examinations on the lungs of mice infected with *C. gattii* R265, *C. gattii* 5815 and *C. neoformans* H99. Our previous investigations indicated that *C. gattii* R265 infected mice showed significantly less macrophage responses than *C. neoformans* infected mice. This meant that *C. gattii* R265 can easily proliferate in alveoli due to poor cryptococcal yeast-cell recognition by macrophages and/or other antigen-presenting cells.[1] To elucidate the mechanism of this phenomenon from the viewpoint of genetic analysis, we performed a microarray assay with gene ontology analysis using the method described earlier.[2]

All the microarray data were deposited in GEO (accession number GSE83594). Although the microarray analysis detected the expression of 45,101 genes, data which contained the "absent" flag were excluded to ensure the quality of data. As referenced in previous investigations, a second selection was then performed using a cut-off value indicating at least a +2.0 fold change ($\log_2$ ratio).

In the present study, 281 genes were specifically upregulated in the lungs of mice infected with *C. gattii* R265 (Fig. 1) in particular phosphoglycerate kinase-1 (PGK1), tenascin C (Tnc) and solute carrier family 7 (Slc7a2) (Fig. 2). Interestingly upregulation of PGK1 also induces cell-cell adhesion in cancer cells.[3] The precise functions of the other upregulated genes should also be investigated. Data obtained from histopathological and molecular investigations can become a linking bridge between histopathological analysis and molecular assay in microbiology. High-virulence *C. gattii* readily resides in the alveoli and this is associated with high upregulation of PGK1. This characteristic may be a candidate for a virulence factor.

Keywords: Cryptococcus gattii, microarray, PGK1

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REFERENCES

1. Okubo Y, Wakayama M, Ohno H, *et al*. Histopathological study of murine pulmonary cryptococcosis induced by *Cryptococcus gattii* and *Cryptococcus neoformans*. Jpn J Infect Dis. 2013; 66: 216-21.
2. Okubo Y, Tochigi N, Wakayama M, *et al*. How histopathology can contribute to an understanding of defense mechanisms against cryptococci. Mediators Inflamm. 2013; 2013: 465319.
3. Wang J, Ying G, Wang J, *et al*. Characterization of phosphoglycerate kinase-1 expression of stromal cells derived from tumor microenvironment in prostate cancer progression. Cancer Res. 2010; 70: 471-80.

Address for correspondence: Yoichiro Okubo, MD, PhD, Department of Pathology, Kanagawa Cancer Center, 2-3-2, Nakao, Asahi-Ku, Yokohama, Kanagawa, 241-8515, Japan. Tel: +81-45-520-2222 (extension number: 5118); Fax: +81-45-520-2202. Email: yoichiro0207@hotmail.com

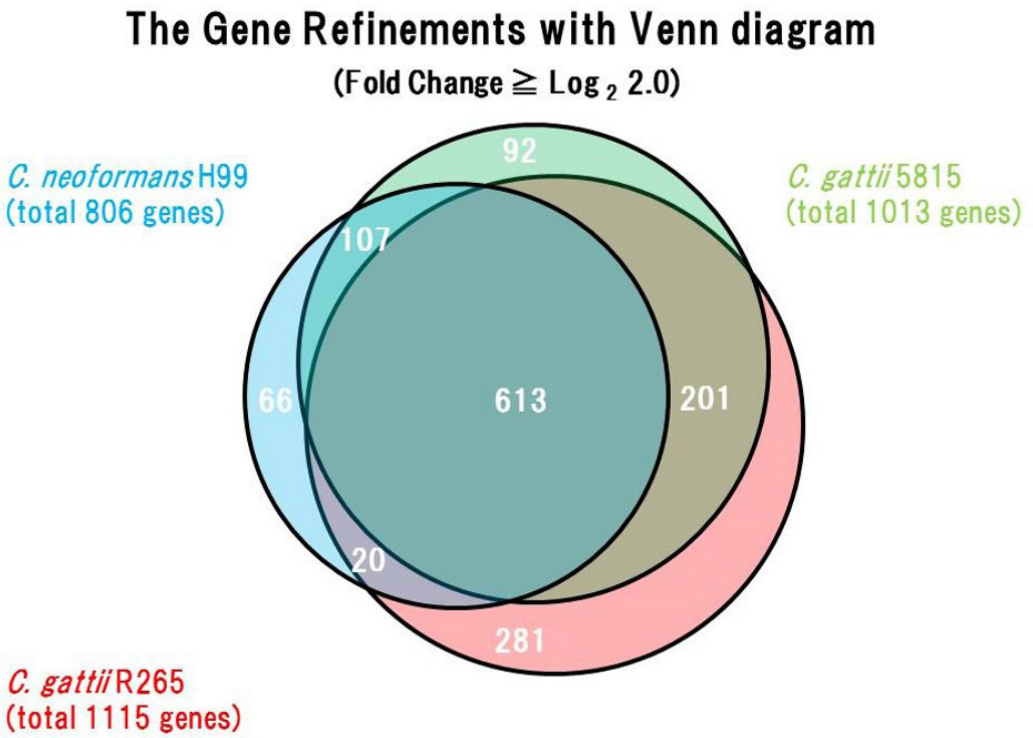

FIG.1: The gene refinements with Venn diagram. Whereas 613 genes were upregulated in the lungs of mice infected with *C. gattii* R265, 5815, and *C. neoformans* H99, commonly, 281 genes were specifically upregulated in the lungs of mice infected *C. gattii* R265

C. gattii R265 infected mice, Fold Change ≧ Log 2 2.0

Fold Change	Gene symbol	Gene name
4.086896	*Pgk1*	3-phosphoglycerate kinase
4.081602	*Tnc*	tenascin C
3.872107	*Slc7a2*	solute carrier family 7
3.501668	*Tfpi2*	tissue factor pathway inhibitor 2
3.413169	*Igf1*	insulin-like growth factor 1
3.384405	*Rgs5*	regulator of G-protein signaling 5
3.33342	*Gla*	galactosidase, alpha
3.318258	*Ccl6*	chemokine (C-C motif) ligand 6
3.199664	*Emilin2*	elastin microfibril interfacer 2
3.190153	*Plau*	plasminogen activator, urokinase

FIG. 2: Gene expression in the mice infected with *Cryptococcus gattii*. The microarray data shows the top 10 upregulated genes in the lungs of mice infected *C. gattii* R265. PGK1 showing the most significant up-regulation in the present study