［課程－２］

審査の結果の要旨

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In this study we tried to elucidate the glutamine induced regulation mechanism using comprehensive genomic and epigenomic analysis. We treated cancer cells with three types of culture medium, including nutrient rich control (control), nutrient starvation (NS), and glutamine supplemented nutrient starvation (NS+Gln) mediums. And by using histone modification ChIP-sequencing and RNA-sequencing analysis, we examined the mechanism of glutamine dependent enhancer activation in cancer cells. We found glutamine supplementation upregulated glutamine responsive gene in HeLa cells comparing to other amino acid supplementation to nutrient starvation condition. These upregulated genes involve various biological processes important for cell proliferation under various stresses such as TCA (tricarboxylic acid cycle), amino acid metabolism, and oxidative stress. Then we found glutamine supplementation partially recovers cell growth comparing to nutrient starvation condition. By global histone modification profiling, we found that glutamine restored 13% of H3K4me3 sites and 6% of H3K27ac sites respectively comparing to nutrient starvation. Our data further indicated that glutamine restored 1553 H3K27ac enhancer cites comparing to nutrient starvation. These H3K27ac enhance sites predicted to be glutamine dependent enhancer sites. Motif search at these predicted glutamine dependent enhancer sites revealed a group of enriched motifs that belong to CNC-family transcription factors (NRF2, BACH1, BACH2, NF-E2, etc.), Fos-family transcription factors (Fosl2, Fra1, Fra2), and Jun-family transcription factors (JunA, JunB, Jun-AP1). Then we examined the top enriched motif, NRF2, from our motif search analysis to elucidate the glutamine induced activation mechanism of this factor. NRF2 was highly expressed at control condition mainly in nucleus, whereas the decrease of expression under nutrient starvation was not recovered by glutamine supplementation HeLa cells treated under control, NS and NS+Gln condition suggested that even though the protein level were not fully recovered by glutamine supplementation. We then tried to elucidate the activation of NRF2 signaling by glutamine using ChIP-sequencing and RNA-sequencing. ChIP-sequencing data showed 215 NRF2 binding peaks were restored by addition of glutamine when compared to nutrient starvation condition. Biological process analysis of nearest genes

that were bound by these 215-glutamine dependent NRF2 binding sites showed mainly NRF2 metabolism related pathways and oxidative stress response pathways. Furthermore, NRF2 strongly bound to subsets of genes that were known targets of NRF2. RNA-sequencing of HeLa cells treated under control, NS and NS+Gln conditions showed a group of genes that were upregulated by glutamine supplementation comparing to nutrient starvation. These genes include various NRF2 targets and involved in NRF2 signaling pathway. In next, we tried to elucidate the co-regulators of NRF2 under NS+Gln condition. Motif analysis of of 215 glutamine-dependent NRF2 binding sites showed mainly CNC-family transcription factors, such as BACH1, BACH2, MAFK, NE-E2. Among them, we examined BACH1 in HeLa cells treated with control, NS and NS+Gln using ChIP-sequencing. We found that there were 569 glutamine dependent BACH1 binding sites. Then we tried to elucidate NRF2/BACH1 regulatory mechanism under glutamine supplemented condition. We found that there were 275 common binding sites of NRF2 and BACH1 that showed different binding signals at NRF2 binding regions under glutamine supplementation. The nearest genes that were bound by these sites showed strong relation to NRF2 regulation pathways, including some of the known NRF2 target genes that appeared to be upregulated by glutamine supplementation comparing to nutrient starvation. In addition, we examined the heterodimeric binding partners of NRF2 and BACH1 known as sMAF (includes MAFF, MAFG, and MAFK). ChIP-sequencing signal distribution of these factors at NRF2_binding regions, NRF_BACH1_common binding regions, and BACH1_binidng regions showed that MAFK mainly distributed at NRF2_BACH1_common binding regions and BACH1_binding regions. And MAFF and MAFG did not show significant differential binding patterns at those regions. We assumed that BACH1 binds to its targets by forming heterodimer with MAFK at its unique binding sites. And NRF2 forms heterodimer with MAFF or MAFG to bind to its target genes. As for the NRF2_BACH1_common binding regions under glutamine supplementation, NRF2 and BACH1 competes with each other to form heterodimer with either one of the sMAFs and then binds to target gene locus. These findings may provide additional insights into NRF2 and BACH1 induced transcriptional regulation that can be translated into clinical studies by developing combined targeted therapies of NRF2 and BACH1 in a nutrient status context, specifically in a context of glutamine availability to cancer cells.

よって本論文は博士（ 医　学 ）の学位請求論文として合格と認められる。