宇宙線によるヒト神経細胞遺伝子の発現

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Gene expression profiles of human neuron-like cells exposed to cosmic rays

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Abstract: Long term manned space missions are planned to implement within the first two decades of the 21st century. A mission of International Space Station (ISS) is in progress, and a plan to explore Moon as well as to visit the Mars is also underway. In space field, the plan will allow space man to live for long terms to do experiments to examine various space sciences. In space environment, 0.2~0.3 mSv a day of radiation exposure will affect cellular metabolisms. As such missions will inevitably result in a significant space radiation exposure on the astronauts, there is an increasing demand to examine their risk. Evaluation of realistic risk associated with the space mission will be in urgent demand to protect them adequately based on both physical and biological knowledge. Considering cellular metabolisms or homeostasis, which affect their risk, examinations of gene expression change would be important. In this study, a human neuron-like cell line, NB-1 was irradiated with 0.1 mGy, 1.0 mGy, 10 mGy, and 100 mGy in single exposure. Thirty minutes and 2 hours after the treatments, mRNA was isolated from the cells and gene expressions were studied with Quantitative PCR analysis. The results showed that the both up- and downregulated gene expressions were seen among mitochondrial genome, electron transport and TCA cycle genes. On the other hand, beta-oxidation, ion transport, ATP production, antioxidative enzymes and factors related gene expressions were downregulated. DNA repair, glycolysis, heat shock protein, and apoptosis related gene expressions were also downregulated except for some genes such as *p53*. The autophagy related gene, and necrosis related gene expressions were also downregulated. These results suggest that the space low-dose irradiation induce intracellular oxidative stress, and may decrease at least partial apoptosis-related gene expression changes, i.e., may increase potential risk of cancer or neuro-degenerative diseases.

宇宙では地球上での被曝に比較し、1 日平均 0.2～0.3mSv の被曝を伴う。我々は、ヒト由来神経様細胞(NB-1)を“通常の培養系”を用い、国際宇宙ステーション JEM に 1ヶ月搭載させる搭載実験を行うことになっている。本研究では、その基礎検討実験として、NB-1 に X 線を低線量照射し、その遺伝子動態を定量 PCR 法により分析した。

方法

ヒト神経細胞株 NB-1 に MBR-1505R (Hitachi Corp.) を用いて 0.1mGy, 1.0mGy, 10mGy および 100mGy の各線量を照射し、30 分後および 2 時間後におけるミトコンドリアゲノム(14 遺伝子)、電子伝達系(14 遺伝子)、TCA サイクル(6 遺伝子)、β酸化(3 遺伝子)、Ion transport(2 遺伝子), ATP 合成(1 遺伝子)、抗酸化酵素(11 遺伝子)、DNA 修復遺伝子(7 遺伝子)、解糖系酵素(25 遺伝子)、ヒートショックタンパク質(30 遺伝子)、細胞死関連(17 遺伝子)の各遺伝子群(計 128 遺伝子)について遺伝子発現変化の解析を定量 PCR 装置 ABI PRISM 7000 (Applied Biosystems)を用いて行った。Table 1 に解析した遺伝子を示す。

結果

ミトコンドリアゲノム、電子伝達系、TCA サイクルでは遺伝子発現が増加傾向を示す遺伝子と減少傾向を示す遺伝子が見られた一方、β酸化、Ion transport, ATP 合成、抗酸化酵素では減少傾向を示す遺伝子群のみが見られた。また、DNA 修復遺伝子、

解糖系酵素、ヒートショックタンパク質、アポトーシス関連遺伝子では *p53* をはじめとする一部の遺伝子発現が増加していたが、全体としては発現減少の傾向を示した。また、アポトーシスとは異なる細胞死型である Autophagy や Necrosis に関連する遺伝子発現も減少していた。抗酸化酵素、DNA 修復遺伝子及び細胞死関連遺伝子の遺伝子発現の増減傾向を Table 2, 3 に示す。

以上の結果、宇宙環境におけるような低線量放射線照射により NB-1 細胞は細胞内酸化ストレスが上昇し、アポトーシス関連遺伝子をはじめとする少なくとも一部の遺伝子発現の減少、すなわち癌化及び神経障害のポテンシャルを増加させるような遺伝子発現変化が認められた(Figure 1)。

Table 1. NB-1 Examine Genes

- Mitochondrial Genome
  nd1, nd2, nd3, nd4, nd4l, nd5, nd6, cox1, cox2, cox3, atpase6, atpase8, cytbm, humanin
- Electron Transport
  atpsyn, cii, coq3, coq7, cytb5, ndufv2, nntm, nuim, sdhb, uqcrc2, uqcrch, uqcrcb, uqcrc1, cyc1
- TCA cycle
  idh2, acon, fumh, mdh2, aldh2, got2
- Beta-oxidation
  mtechb, hada, mtsceh
- Ion transport
  nhe6, frataxin
- ATP production
  mtck
- DNA Repair
  p53, atm, dnapk, ku86, brca1, udg1, ogg1
- Apoptosis related
  ant1, ant2, aif, casp8, casp9, apaf1, bax, bcl2, casp3, mnsod, p53, vdac1, vdac2, vdac3, cyt-c
- Necrosis
  cyp-40
- Autophagy
  becn1
- Glycolysis
  aldo a, aldo b, aldo c, bpgm, dlat, dld, eno1, eno2, eno3, gapds, gpi, hk1, hk2, ldhb, pdha, pfkl, pfkm, pgam1, pgam2, pgk1, pgm1, pkm2, suclg1, suclg2, tpi1
- Antioxidative Enzymes・Factors
  ho-1, ho-2, transferrin, ferritin, nrf1, nfe2l1, nrf2, keap1, fech, cuzn-sod, mnsod
- Heat Shock Protein
  hspa8, hspa1(hsp70i), hsp47, hspa9(mthsp70), hspd1(mtmp), hspa5, hspa6, hspa2, hspa4l, hspa4, hspa14, stch, hsp90α, hsp90β, tra1, hsp105, bip, djb1, tid, dna J, djb 4, dnaj 3, hsj1, tcp20, cctb, chaperonin 10, tcomp1, cctd, ccth, hspa1l(hsp72)

Table 2. Gene Expression Changes by Quantitative PCR. Antioxidative enzymes・factors and DNA Repair

Antioxidative enzymes・factors	0.1mGy	1mGy	10mGy	100mGy
ho-1	↑↓	↓↓	↑↑	↑↑
ho-2	⇩⇩	↓↓	↑↑	↑⇩
transferrin	⇩↓	↑↓	↓↓	↑↑
ferritin	↓↑	↑↑	↑↑	↑↑
nrf1	⇩⇩	↑↓	↑↑	↑↓
nfe2l1	⇩⇩	⇩↓	↓↑	↓↑
nrf2	↓↓	↓↓	↑↓	↓↓
keap1	↓↓	↓⇩	↑↑	↓↓
fech	⇩↓	↓↑	↑↑	↑↑
cuzn-sod	↓↓	↓↓	↑↑	↑↑
mnsod	⇩↓	↓↑	⇩⇩	⇩⇩

DNA repair	0.1mGy	1mGy	10mGy	100mGy
p53	⇩⇩	↑↑	⇧⇧	⇧⇧
atm	↓↑	↑↓	↓↓	↑↑
dnapk	↑↑	↓↓	↑↑	↑↑
ku86	↑↑	↑↑	↓↓	⇩↓
brca1	⇩↓	⇩↓	↓↓	⇩↓
udg1	↑↑	↑↑	↓↓	↑↑
ogg1	↓↓	⇩↓	⇩⇩	↓⇩

↑↓ 0.5h 2h
↑ : up
↓ : down
⇧ : up (p<0.05)
⇩ : down (p<0.05)
↑↑ : up (0.5h,2h)
↓↓ : down (0.5h,2h)

Table 3. Gene Expression Changes by Quantitative PCR. Cell Death related genes

Cell Death related	0.1mGy	1mGy	10mGy	100mGy
cyp-40	⇩⇩	⇩⇩	↓⇩	⇩⇩
becn1	↓↓	↓↓	⇩⇩	⇩⇩
ant1	⇩⇩	↑↓	↓↓	↑↓
ant2	↓⇩	↓↓	↑↑	↓↑
aif	⇩⇩	↓↓	⇩⇩	⇩⇩
caspase8	↓↑	↓↓	↓↓	↓⇩
caspase9	↓↓	↓↑	↑↑	↑↑
apaf1	⇩↓	↓↓	⇩⇩	↑↓
bax	↓↓	↓⇩	⇩⇩	⇩⇩
bcl2	↓↓	↑↓	↓↓	↑↑
casp3	⇩↓	↓⇩	↑↑	⇧↑
mnsod	⇩↓	↓↑	⇩⇩	⇩⇩
p53	⇩⇩	↑↑	⇧⇧	⇧⇧
vdac1	⇩⇩	↓↓	↓⇩	⇩⇩
vdac2	↑↑	↓↓	↓↑	↓↓
vdac3	⇩⇩	↑↓	↓↓	↓⇩
cyt-c	⇩⇩	↓↑	⇩⇩	⇩⇩

↑↓ 0.5h 2h
↑ : up
↓ : down
⇧ : up (p<0.05)
⇩ : down (p<0.05)
↑↑ : up (0.5h,2h)
↓↓ : down (0.5h,2h)

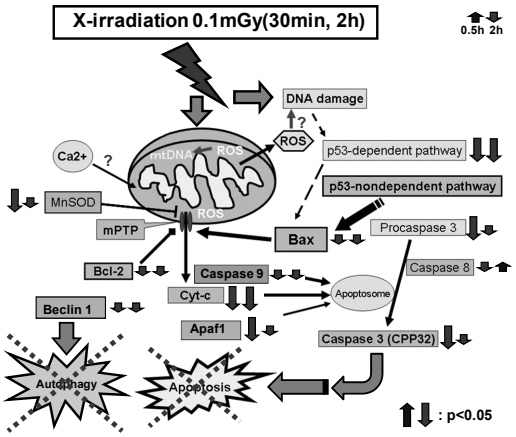

Figure 1. Cell Death related Gene Expression Changes by 0.1mGy X-irradiation.