Research on proliferation inhibition V79 cells and FD-LSC-1 cells in deep underground (rock cover 1470m) environment and its change profile based on transcriptomics and proteomics

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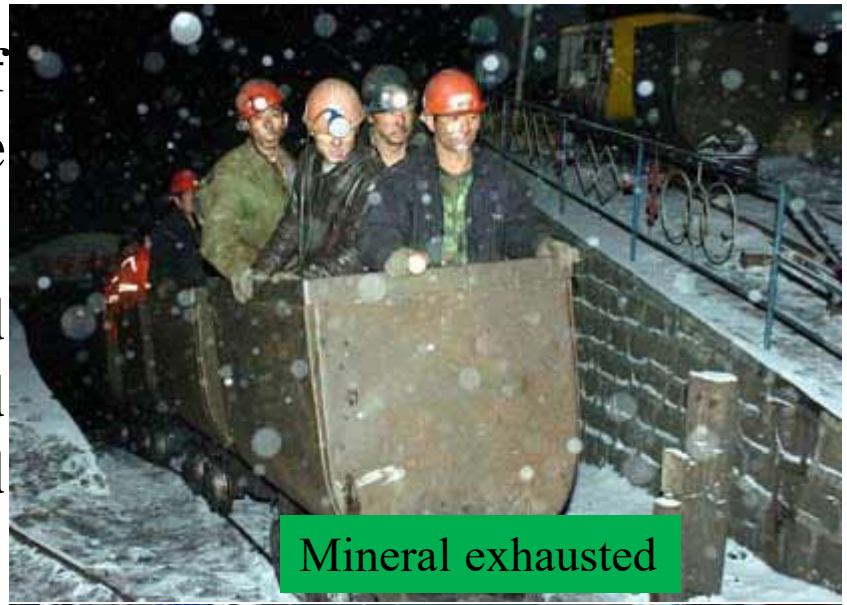
Summary

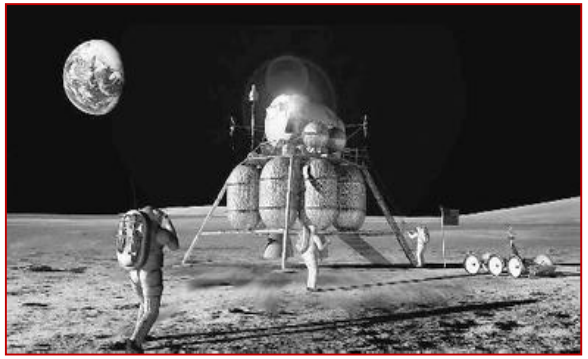
Background

- **Resources in the shallow depths of the earth have gradually become exhausted.**
- **Therefore, history has progressed to a new era of exploring and exploiting the deep-underground space.**
- **Importantly, utilizing the deep-underground space to augment natural resources is a cheaper and less complex solution than moving to another planet.**



Background

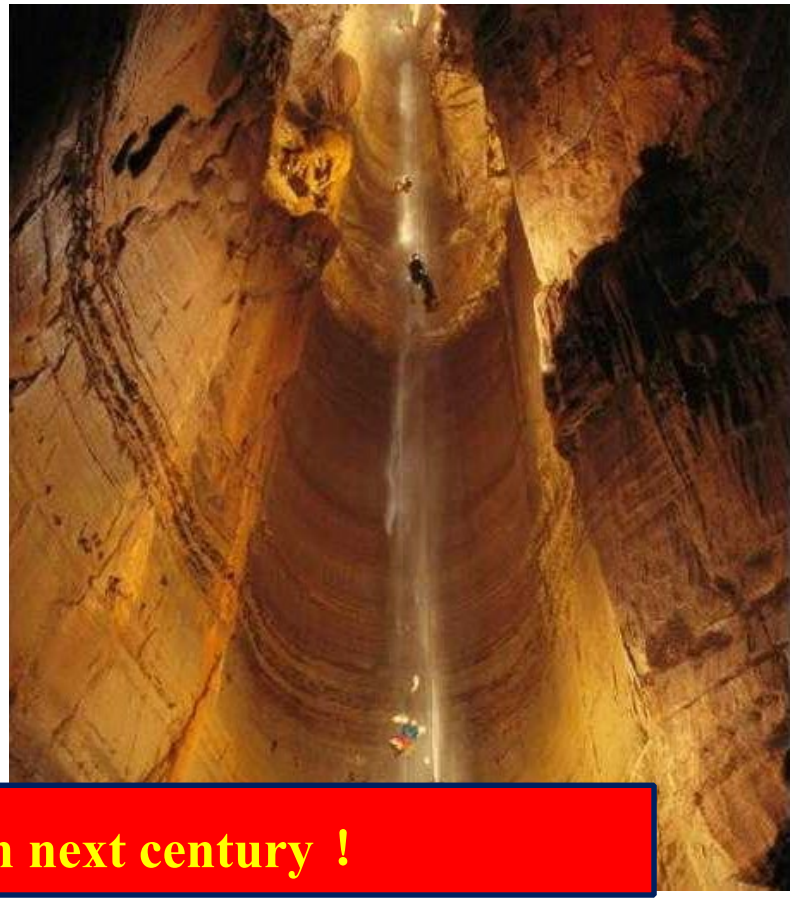
- ✓ In 1991, the “Declaration of Tokyo,” drafted at the International Conference on Urban Underground Space, stated that the”21st century is the century of developing and utilizing underground space”.



- ✓ In 2016, exploiting the deep underground space and its resources has become a national priority for the future development of science and technology in China.

Background

	At present	In 2030
• Coal mining:	1500m →	3000m
• Metal mining:	4800m →	3000m
• Geothermal exploitation:	5000m →	6000m
• oil and gas exploitation:	7500m →	10000m
• Underground detect :	7000m →	15000m



1/3 people would live or work underground in next century !

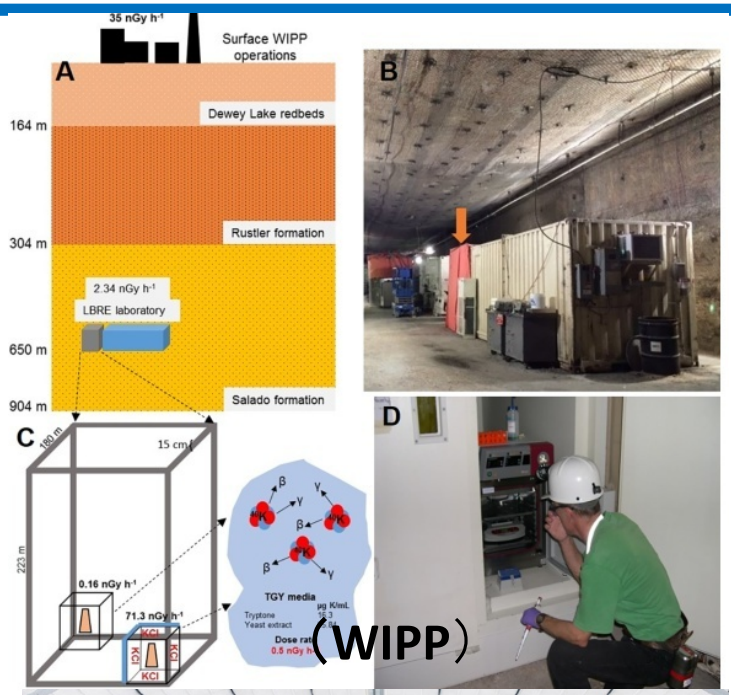
Xie H, et al. Quantitative definition and investigation of deep mining. J China Coal Soc.2015

Xie H. Research framework and anticipated results of deep rock mechanics and mining t. ; heory. Adv Eng Sci.2017

Pathegama G et al. Opportunities and Challenges in Deep Mining: A Brief ReviewEngineering . 2017

History and location of biological research in deep-underground laboratories

- 1964 Swiss（SIMP ）
- 1987 France （CNRS ）
- 1995 Italy （LNGS）
- 2009 USA （WIPP）
- 2016 France（Modan）
- 2017 China （CJEM）
- 2017 Canada（SNOLAB , in plan ）

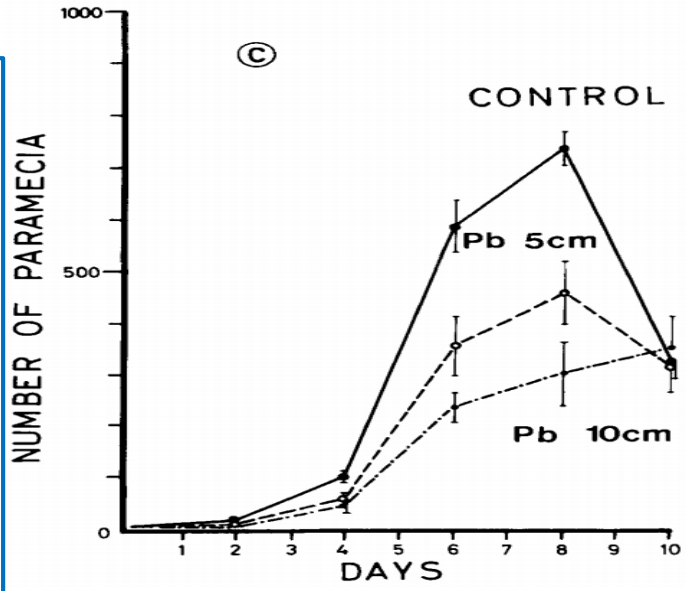


（WIPP）

（LNGS）

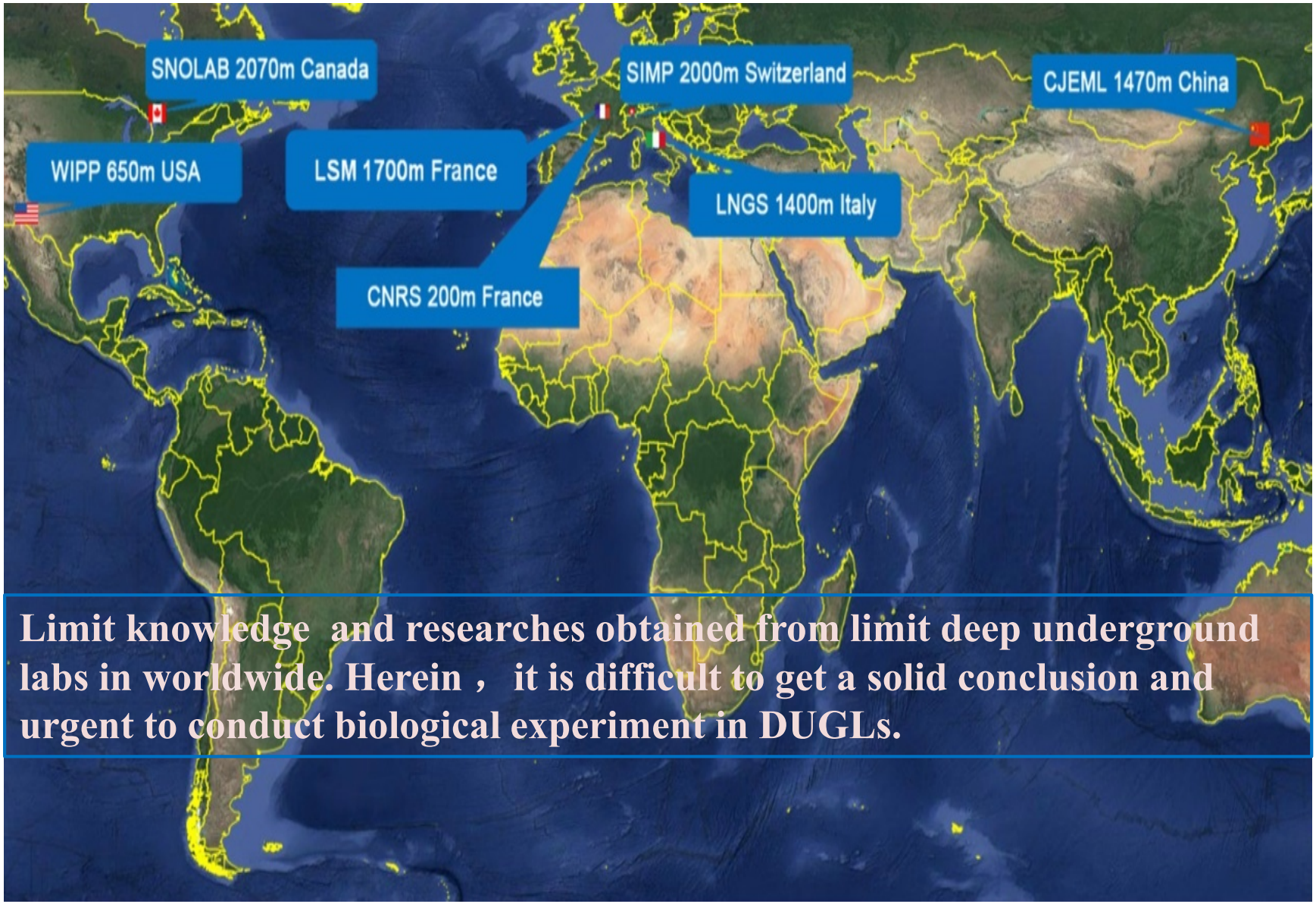
Advancements in biological research in deep-underground laboratories

- **Planel et al. (1987) discovered that the number of cells was reduced and the generation time was increased in *Paramecium tetraurelia* cultures grown in CNRS ; this result was verified by many researchers .**
- **However, studies of growth rates in other mammalian cells TK6 cells and V79 cells, have not indicated a clear difference in growth rates between cells grown in a control environment and at LNGS after several months in culture.**
- **Castillo et al. were unable to replicate their original experiments that showed reduced growth rates *in S. oneidensis* cultures at the WIPP; although, growth rates in *D.radiodurans* cultures were reduced within 24h of being introduced to the WIPP compared to parallel populations grown in the same under-ground laboratory with a simulated standard background environment.**



Planel H 1987. Health physics

Worldwide locations of deep-underground laboratories focusing on biological research



Limit knowledge and researches obtained from limit deep underground labs in worldwide. Herein， it is difficult to get a solid conclusion and urgent to conduct biological experiment in DUGLs.

Background

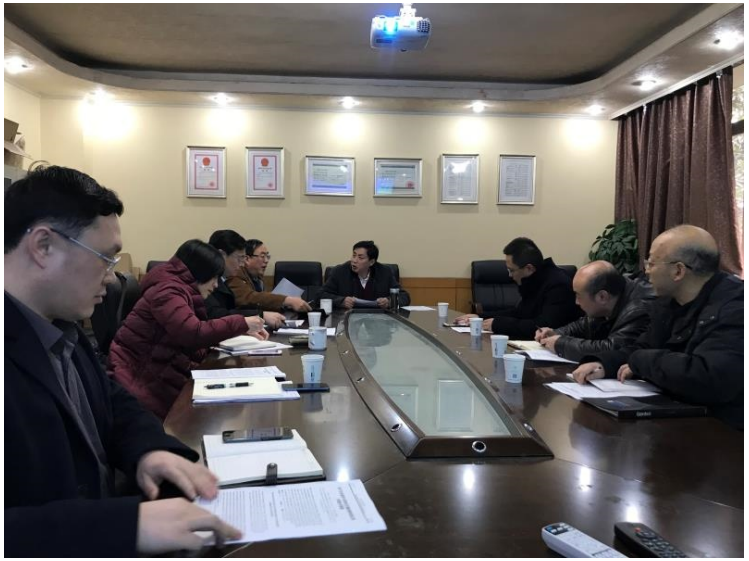
•Besides, the environmental factors in the deep-underground (e.g. low background radiation, rock, humidity, temperature, isolation, microorganisms and other unknown factors) might have biological effects on humans and other organisms.

•However, little is known about the effect of these factors on the health of humans or other organisms that work or live in the deep-underground space, even the nature of the deep Earth itself.

•Fortunately, biological effect of deep underground environment arises the researchers interest.

Background

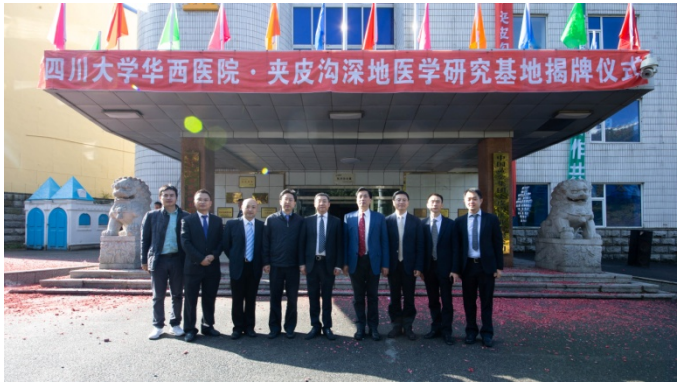
- **Heping Xie, academician of the Chinese Academy of Engineering, advocated the need to further explore coping strategies in organisms in response to harmful factors in the deep-underground, but also emphasized the value of developing methods to efficiently and safely harness the beneficial elements of the deep-underground.**
- **Consequently, Prof. Xie highlighted the important role of the deep-underground in medical research and proposed a multidisciplinary approach to medical research in the deep-underground.**
- **In 2015, Prof. Xie assembled the Deep-underground Medicine (DUGM) team.**



Background

In 2018 , pro. Xie and his DUGM team defined the DUGM as an multi-disciplines: aiming to systematically research the effects of the environmental factors in the deep underground on the physiology, psychology (for organisms with cognition), and pathology of living organisms, and the underlying mechanisms, and explore coping strategies in response to harmful factors , as well as methods to efficiently and safely exploit the beneficial elements of the deep-underground.

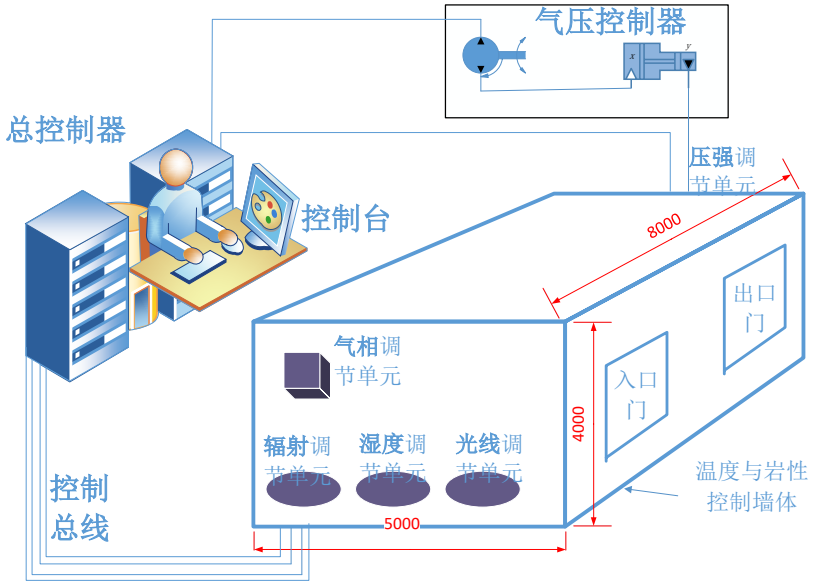
Therefore, a real deep underground lab(DUGL) and basis focusing on medical research were constructed in the northeast of China. And now, we present our research conducted in the DUGL.



Background

As the deepest underground lab，China Jinping lab（CJPL）will been a comprehensive lab including medical research.

Also, a simulation cabin of DUGM will been constructed in Sichuan university.



Summary

1 Background

2 Method

3 Result

4 Discussion

5 Conclusion

Method(location of DUGL)

Erdaogou Mine, China National Gold Group Corporation Jiapigou （CJEM）locates in northeast of China, and about 3000 km from Sichuan university.

One of the deepest mines in China, reaching a mining depth of approximately 1500 m.

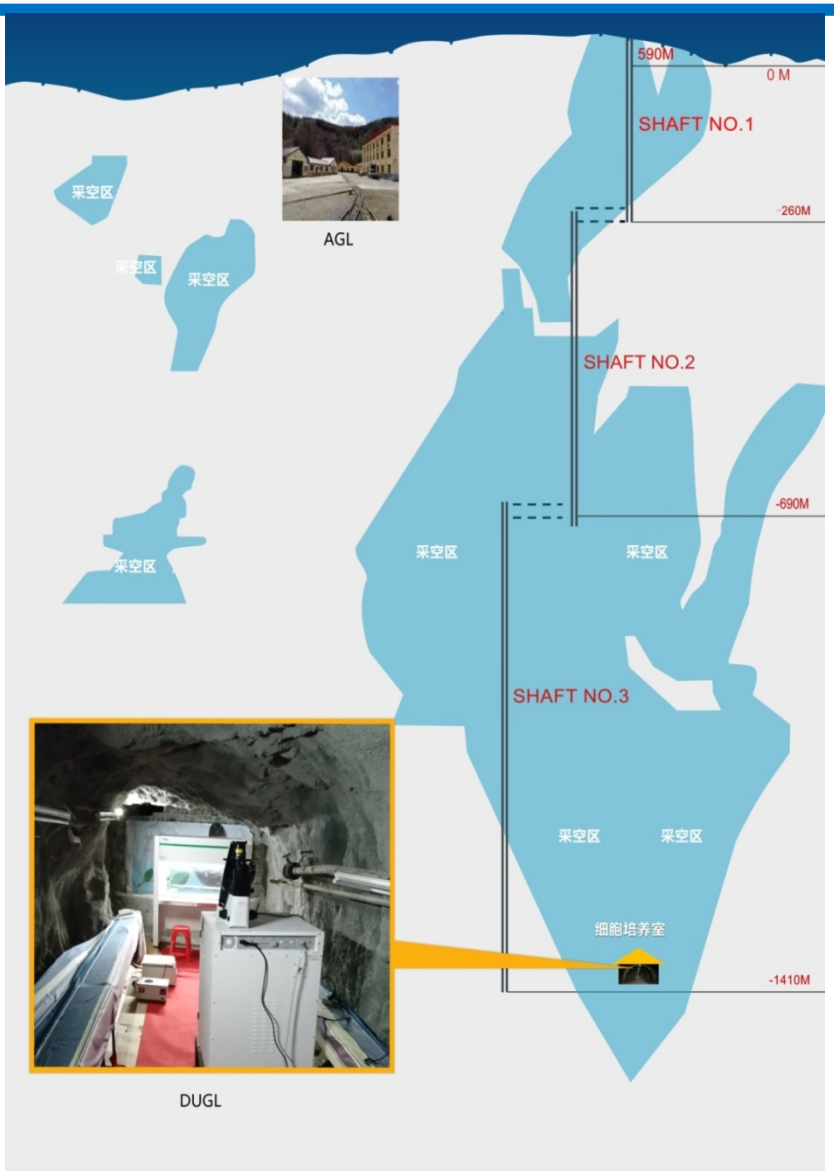
As an initial project, a deep-underground cell culture laboratory (DUGL) funded by the West China Hospital of Sichuan University was set up in a goaf that is under 1470 m of rock and 820 m below sea level.

Method(location of DUGL)

Control experiments are conducted in an aboveground laboratory in an office building near the entrance of the mine (AGL) .

To reach the DUGL, we need walk about 1600m, and take three elevators from the entrance of CJEM to DUGL. The total time was about 1.5-2 hours .

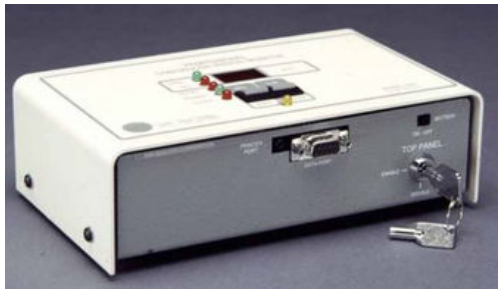
In fact, it is a very hard work conducting biological experiment in the lab.



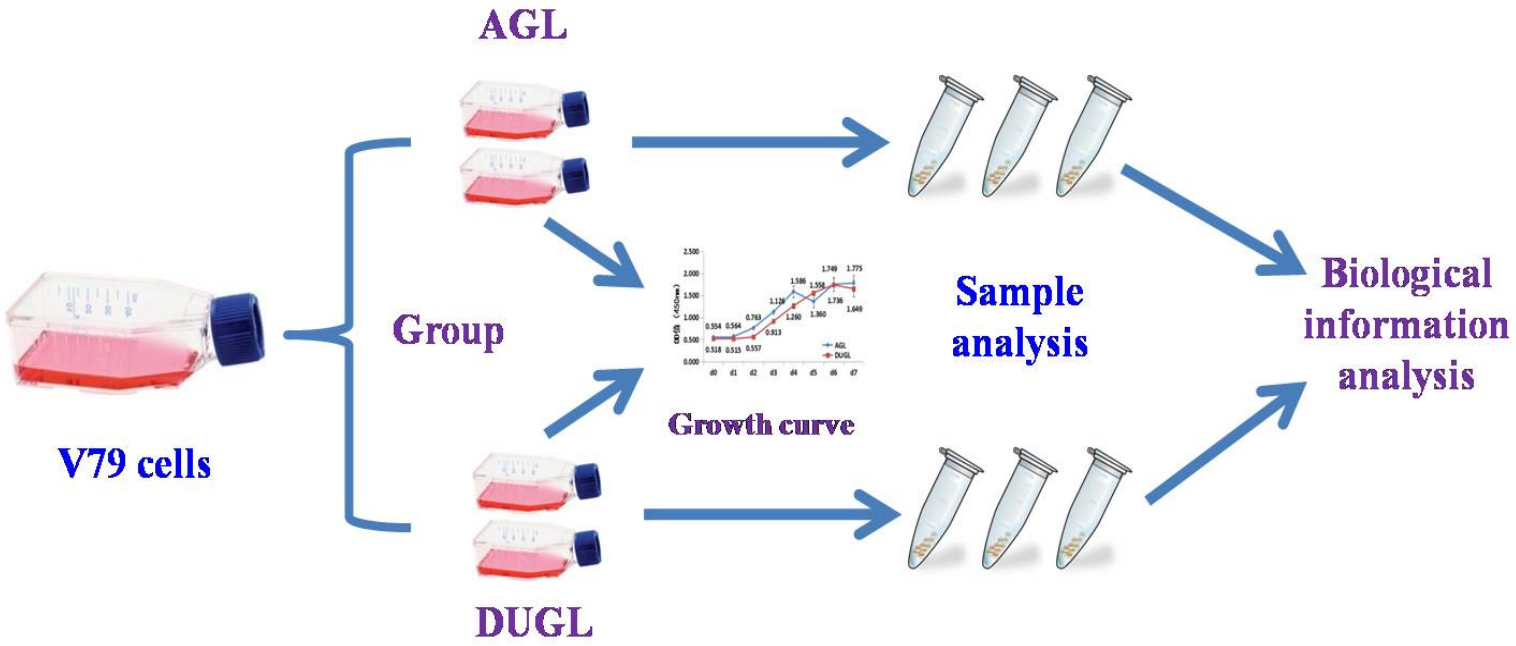
Measurement of environmental parameters

Total dose rate of γ rays	**ATOMTEX， Belarus**
CO_2, air pressure and relative humidity	**Testo480, Testo, Germany**
O_2	**AR8100, SIGMA ,China**
Radon gas	**Sunnuclear， USA**

The points monitored were all at 1m to incubators, 0.5m to ground and 0.3m to palisades/wall.



An overview of the experiment



CCK-8 for growth curve、 Transmission Electron Microscopy （V79 2d,FD 4d）, RNA extraction（ V 79 2d,FD 4d）,protein extraction （V79 2d,FD 4d ） ,PCR verification for RNA, PRM verification for protein.

Cell culture

Chinese hamster V79 lung fibroblast cells (Shanghai Enzyme-linked Biotechnology, China) and laryngeal squamous cell carcinoma FD-LSC-1 (FD-LSC-1) cultured in Dulbecco's modified eagle medium (DMEM) (Gibco), supplemented with 10 % foetal calf serum(GEMINI), 50 U dm $^{-3}$ penicillin and treptomycin(Gibco).

The frozen cells were resuscitated. When the cells sate was well and cell attachment rate was over 80%, the cells passage were conducted And then the cultures were divided into four bottles .

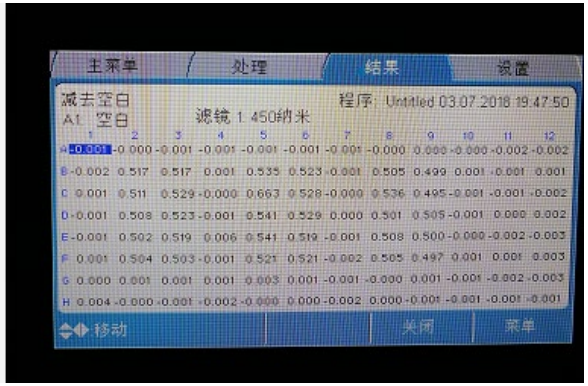
Two of them were randomly selected and respectively cultured at DUGL and AGL.

Sample extraction

- **V79 cells cultured 2 days in DUGL, FD-LSC-1 cultured 4 days in DUGL .**
- **1ml Trizol was added to the cells(n=3).**
- **Cells were fixed with 2.5% glutaraldehyde for Transmission Electron Microscopy scanning.**

Growth curve

Cell suspension was inoculated into 96-well plates in the AGL and DUGL respectively (5×10^{5}cells/ml,200μl/well). Five duplicate wells were measured each day. The plates were cultured at 37℃with 5% CO_2. 10ul Cell Counting Kit-8(CCK-8) (MCE,U SA) was added to the well of the 96-well plates every day, and then the 96-well plate were incubated 4 hours at 37 ℃ with 5% CO_2.Then,the light absorption *value(OD_{450} nm)* were measured.



Transmission Electron Microscopy(TEM)

•V79 cells grow two days in AGL and DUGL respectively were fixed with 2.5% glutaraldehyde. And then pre-chilled PBS buffer was added to the cells after remove of the glutaraldehyde, stored at 4℃ for 20 min. This step was repeated for five times. After that, the cells were fixed with 1% OsO_4 for 5 hours at 4℃. Remove of OsO_4, cells were submitted to dehydration in a graded series of ethanol(30%、50%、70%、90%、100%). Cells were then embedded in 6189 Epoxy resin, and haff -thin sections of 80 nm were cut. After location on haff-thin sections, the super-thin section were cut and finally stained with 3% uranyl acetate and lead citrate for observation under a HITACHI H7650 TEM.

RNA preparation,quality controlling and cDNA synthesis

After the cells grown two days in AGL and DUGL respectively, the samples extracted by Trizol reagent was used to RNA sequencing. It's purity and integrity was tested by 1% agarose gel electrophoresis(Sigma-Aldrich). And then Agilent 2100 Bioanalyzer(Agilent Technologies, Palo Alto, CA, USA) was used to determine RNA integrity, NanoDrop (NanoDrop Technologies,Inc. Wilmington, DE, USA) was used to measured RNA quantity. The RNA integrity number (RIN) was more than 7.0 . Ribo-Zero™ GoldKits (Epicentre, USA) were used to remove the rRNA.

RNA-sqe and data processing

•3 µg RNA from each sample was used for cDNA library construction. NovaSeq 6000 Illumina sequencing system (Illumina, San Diego, CA,USA) was used for sequencing.

• The sequencing data was analyzed using CASAVA software for base calling; raw data were transformed into fastqstored(FASTQ) documents and the low-quality base data was removed.

• HiSAT2 software was used to aligned sequencing data according to the reference hamster genome (GCA_000223135.1, GenBank assembly accession).

•Read Count for each gene in each sample is counted by HTSeq, and Fragments Per Kilobase Millon Mapped Reads (FPKM) were then calculated to represent the expression level of genes and lnc RNA in each sample.

TMT Protein Quantification

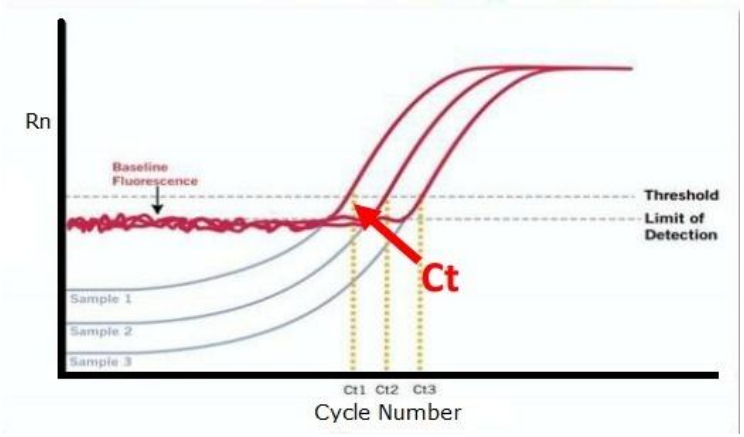
- Protein lysis was added to residual samples after RNA extraction of cells.
- the lysates were sonicated on ice . The protein concentrations were analyzed by the bicinchoninic acid (BCA) Protein Assay kit (Fisher Scientific, USA).
- TMT mass spectrometric analysis.
- The raw data was processed and selected by Proteome Discoverer(PD)(Version 1.4.0.288, Thermo Fisher Scientific, USA).
- Protein identification were searched using MASCOT version(Version 2.3.2 , Matrix Science).
- After that, according to uniquely identified peptides that belong to the specific individual protein, the relative quantification of identified proteins was calculated.

Bioinformatics analysis

- **The differential expression(DE) RNAs were selected (|log2Ratio|≥1 and q≤0.05), along with p *p*<0.05.**
- **According to the *P*-value≤0.05 and fold change ≥1.5 after T test, protein were determined as significantly differentially.**
- **GO enrichment and KEGG pathway were applied on these differentially expressed RNAs and proteins.**

qRT-PCR Verification for DE RNAs

- 19 DE RNAs of V79 cells and 22 DE RNAs of FD-LSC-1 were selected for verification of qRT-PCR.
- Total RNA extracted was reverse transcribed for cDNA synthesis using a PrimeScript RT Re-agent Kit with gDNA Eraser (RR047A,Takara, Japan).The Primer-BLAST(https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi) of NCBI was used for primer design.
- 7500 Real-time PCR system(Applied Biosystems, Foster City, CA, USA) was used for Real-time Quantitative polymerase chain reaction (qRT-PCR).
- Three biological repeats were applied in each experimental group. A value of $p \leq 0.05$ was considered a significant difference.



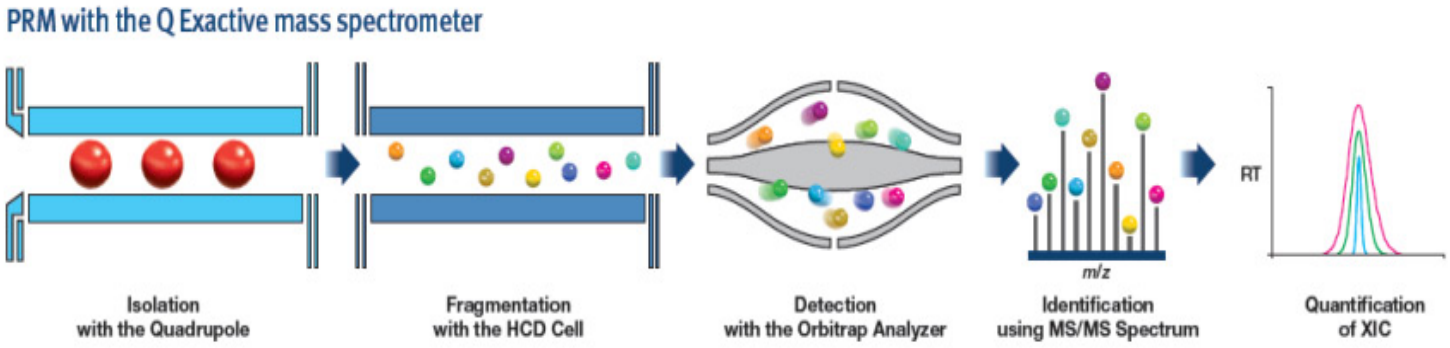
Verification by Parallel Reaction Monitoring

Parallel Reaction Monitoring（PRM）used for verification of the TMT results was performed on Triple TOF 6600+ LC-MS/MS system.

The process of protein extraction, quality control, lysis and desalt was same as the TMT experiments. The DDA raw files were analyzed with MaxQuant (version 1.3.0.5) software using default settings.

The data were input into Protein Pilot software for searching against the UniProt-cricetulus+griseus. fasta database.

The PRM validation data was input into Skyline; peak shapes for target peptides were manually checked.



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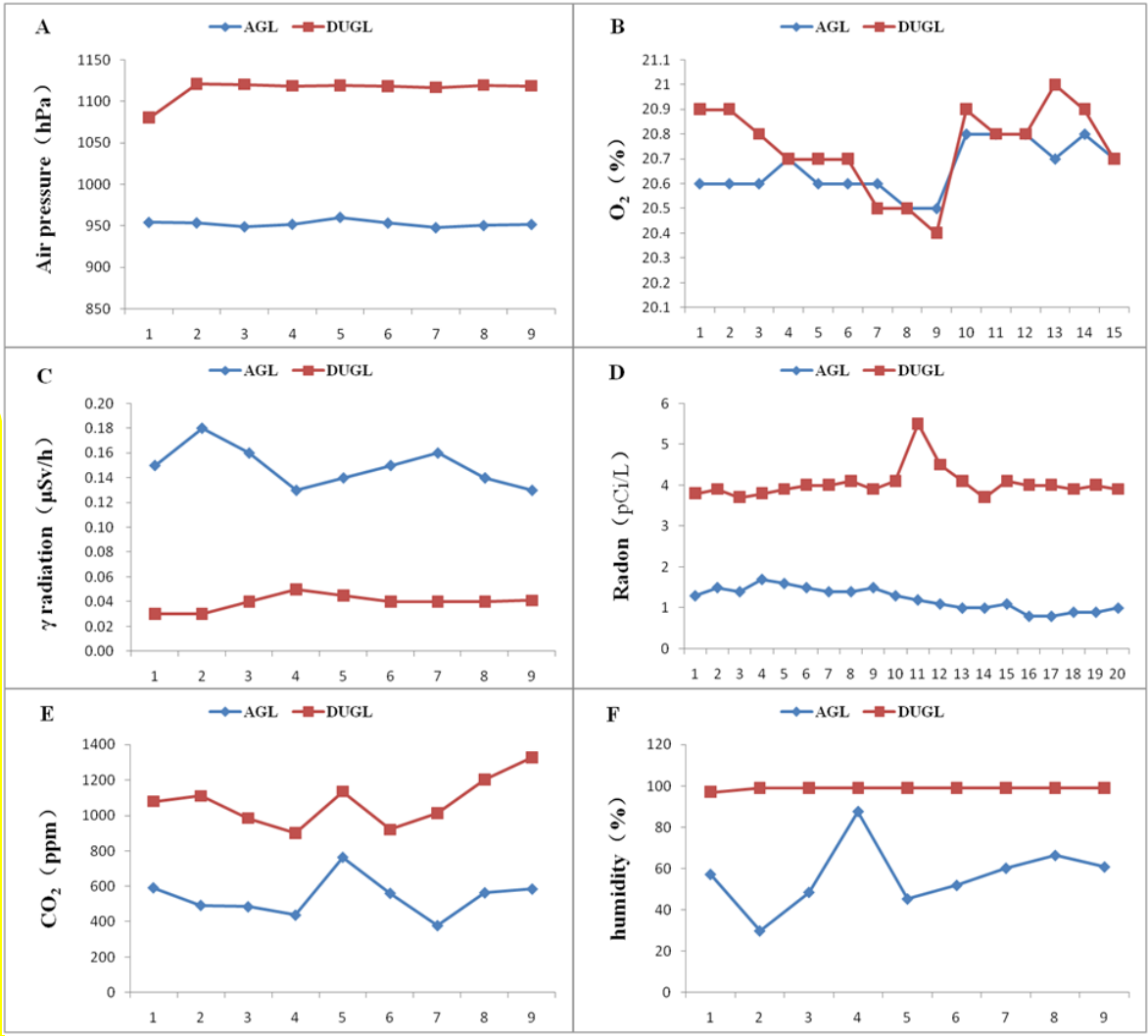
Result(Environmental parameters of AGL and DUGL)

Environmental parameters	AGL	DUGL	*p*
Air pressure(hPa)	951.9(949.65-953.9)	1118.2(1117.3-1119.6)	<0.001
O_2 concentration (%)	20.6(20.6-20.8)	20.8(20.7-20.9)	0.079
Total γ radiation dose rate(μSv/h)	0.15(0.13-0.18)	0.04(0.035-0.045)	0.005
Radon concentration (pCi/L)	1.25(1-1.47)	4.0(3.9-4.1,3.7-5.5)	<0.001
CO_2 concentration (ppm)	540.11±110.39	951.9±137.56	<0.001
Relative humidity (%)	57.2(46.9-63.6)	99(99-99)	<0.001

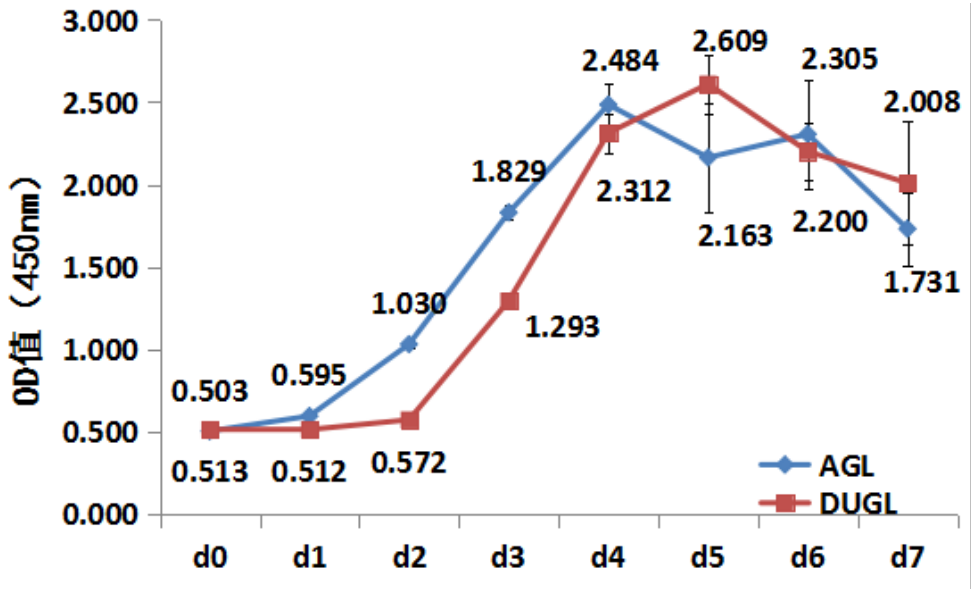
Note : deep-underground laboratories(DUGL), above-ground laboratory (AGL); $\bar{\chi}\pm S$, *M(Quartile)*.

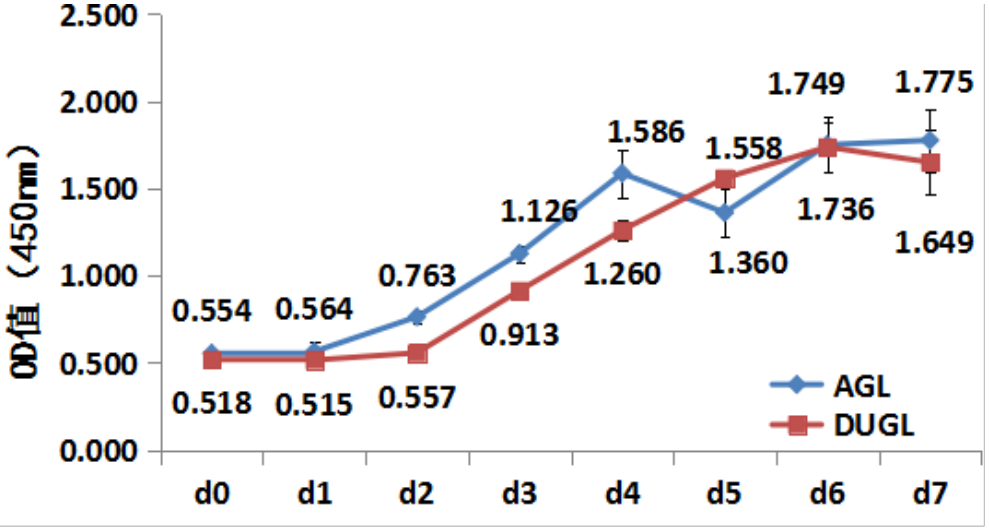
There was no statistical difference in O_2 concentration between DUGL and AGL. The total dose rate of γ rays in the DUGL was significantly lower than AGL (*p=0.005*), while the humidity (*p*<0.001), air pressure (*p*<0.001), concentration of CO_2 and radon gas (*p*<0.001) were all higher than AGL.



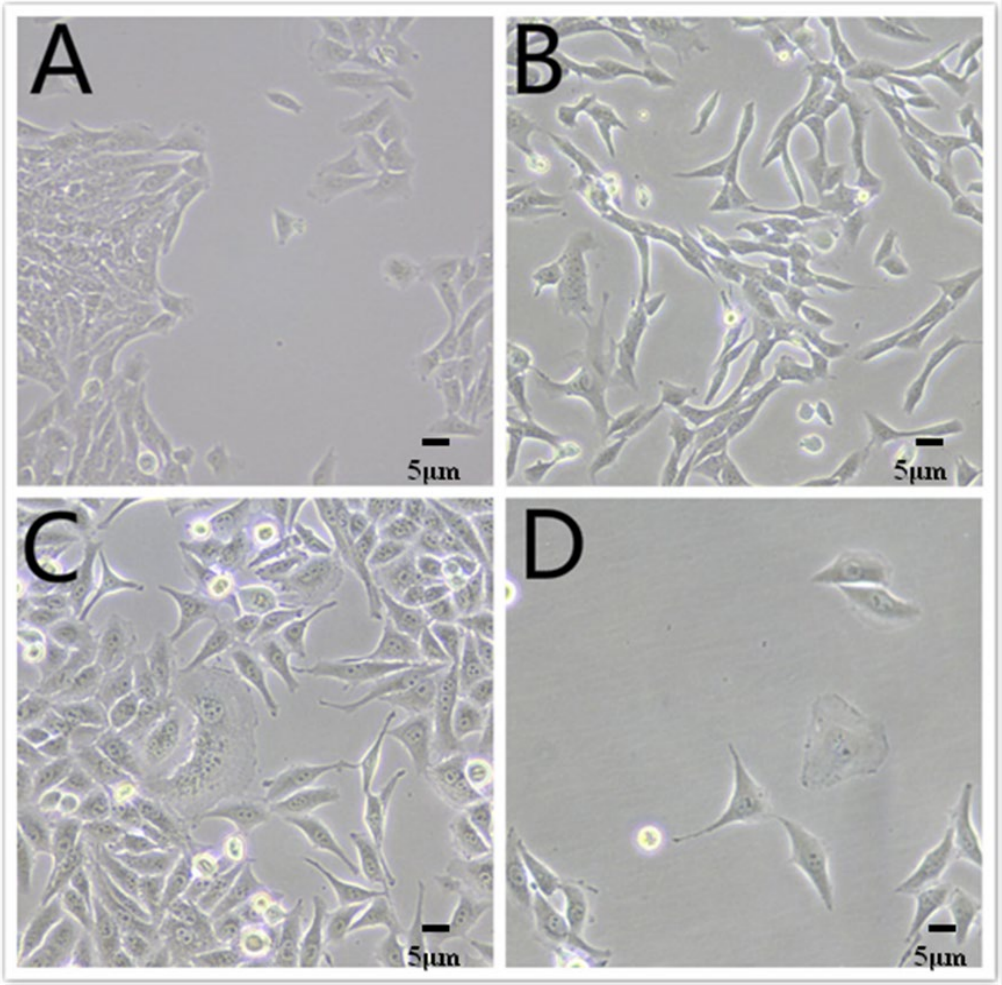
Growth curve

CCK8 assay results from the 5 repeats of V79 and FD-LSC-1cells with 7-day indicated that the proliferation rates of cells grown in the DUGL environment were slower than in the AGL.





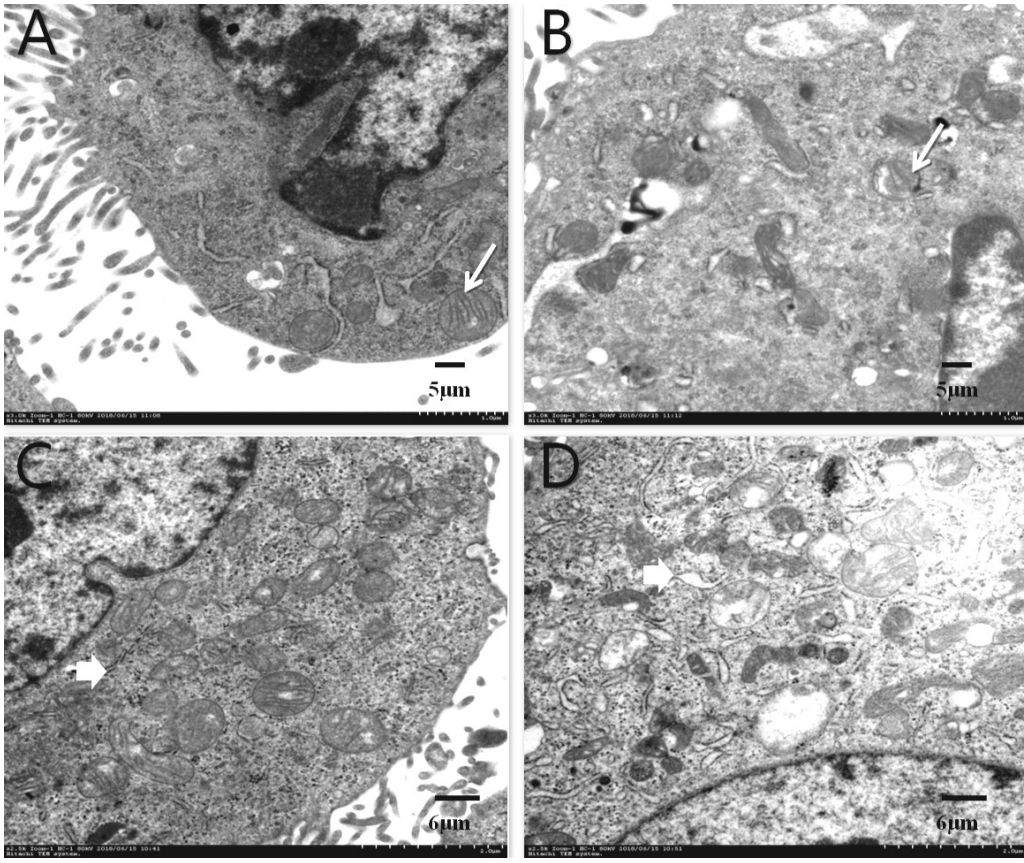
Result（microscopic image of cells cultued two days ）



A . V79 of AGL, B. V79 of DUGL, C. FD-LSC-1 of AGL, D. FD-LSC-1 of DUGL ; these were less cells in group of DUGL compared with AGL

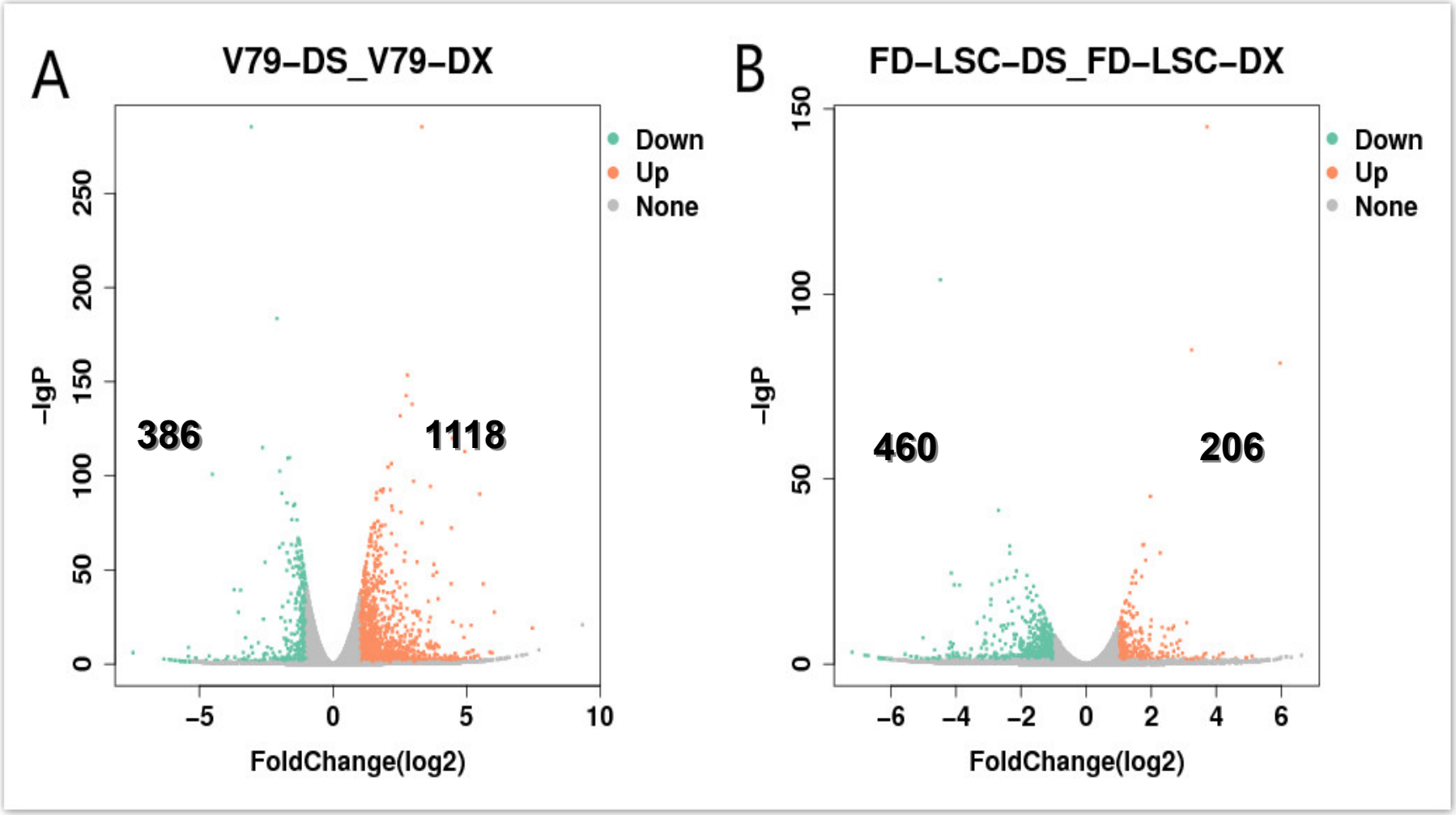
Result（TEM image of cells cultued two days ）

The features of transmission electron microscopy of V79 and FD-LSC-1 cells cultured in DUGL were agglutination of nuclear heterochromatin for both V79 and FD-LSC-1 cells, decrease or disappearance of mitochondria crista in V79 cells, and abundant rough surfaced endoplasmic reticulum and enlarged cisternae.



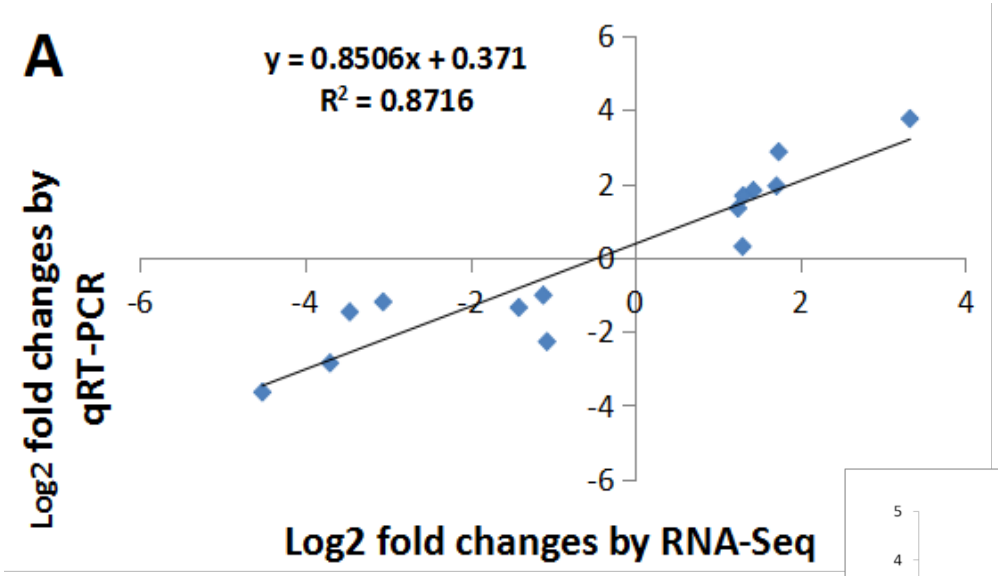
A，V7 9 cells of AGL ,3000×； B， V7 9 cells of DUGL 3000×； C, FD-LSC-1 cell of AGL 2500×； D， FD-LSC-1 cell of DUGL2500×。

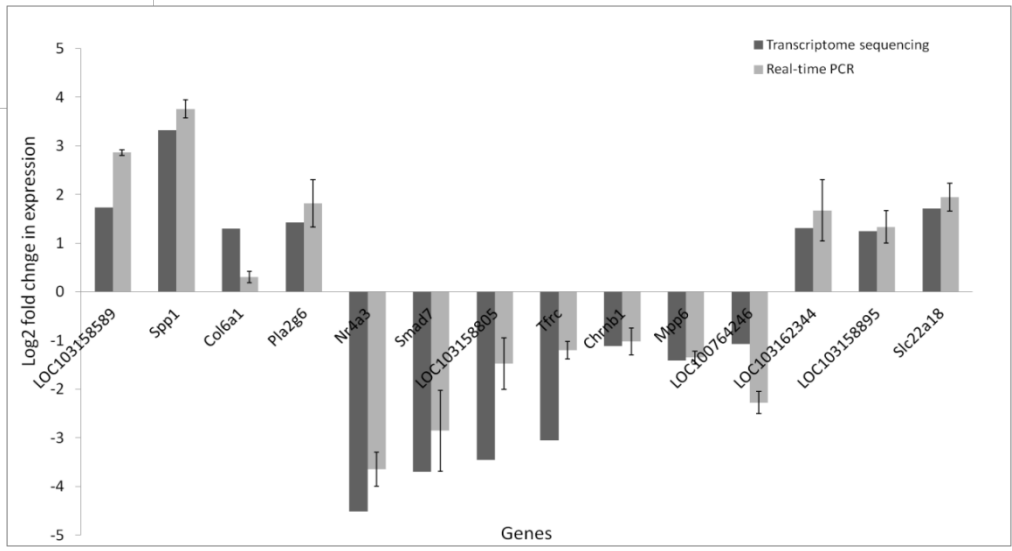
Result （valcono plot of DE RNAs）



DS=AGL, DX= DUGL

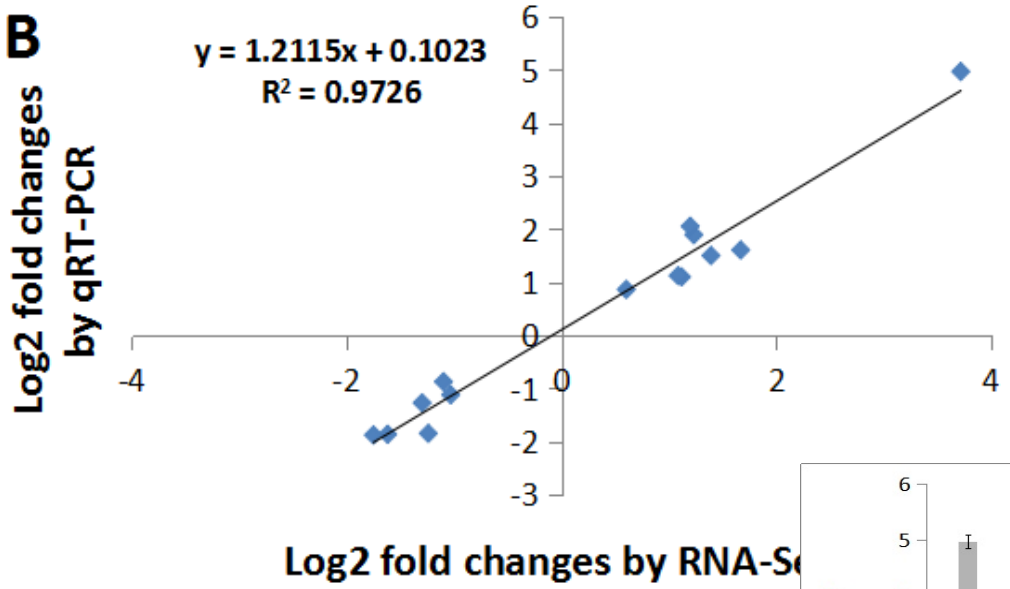
Result（V79 verification of mRNAs）

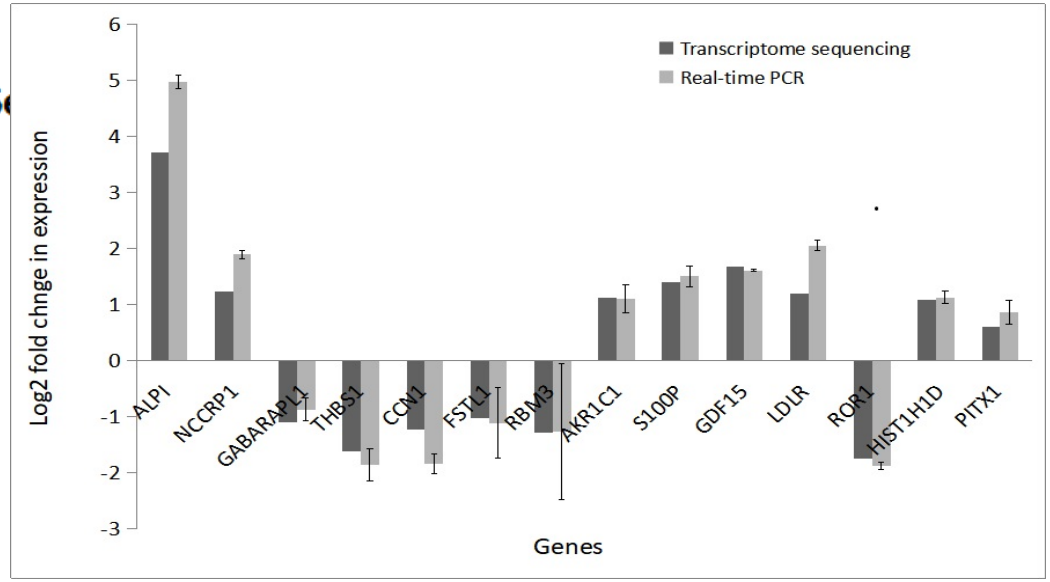




14 mRNAs of 19 DE mRNA selected had the commence expression patterns， The correlation coefficient were 0.87.

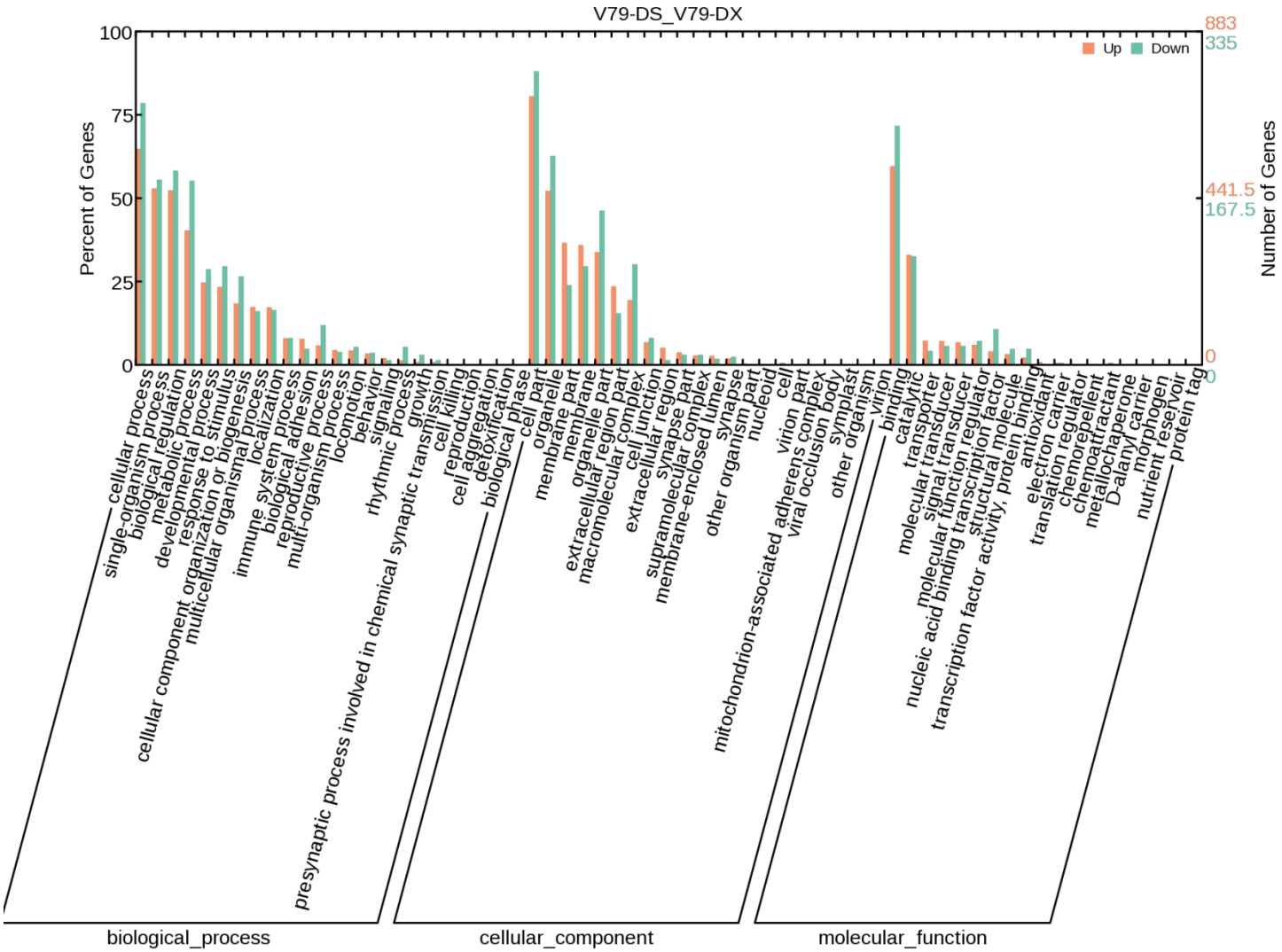
Result（FD-LSC-1 verification of mRNAs）



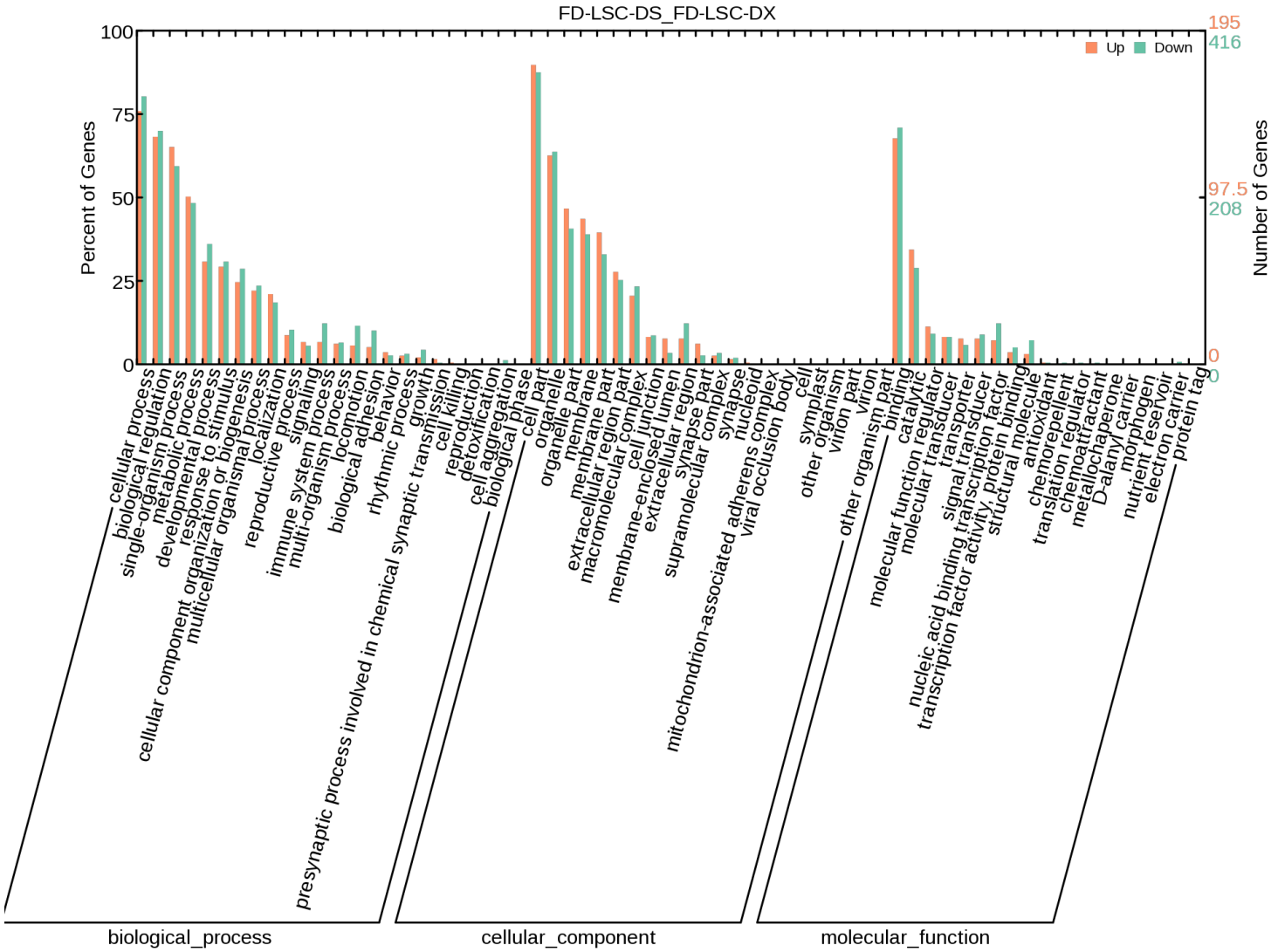


16 mRNAs of 20 DE mRNA selected had the commence expression patterns ,the correlation coefficient were 0.976.

Result （V79 GO analysis of DE RNAs）



Result (FD-LSC-1 GO analysis of mRNAs)



Result （V79 analysis of KEGG）

V79 DE mRNAs KEGG pathway:

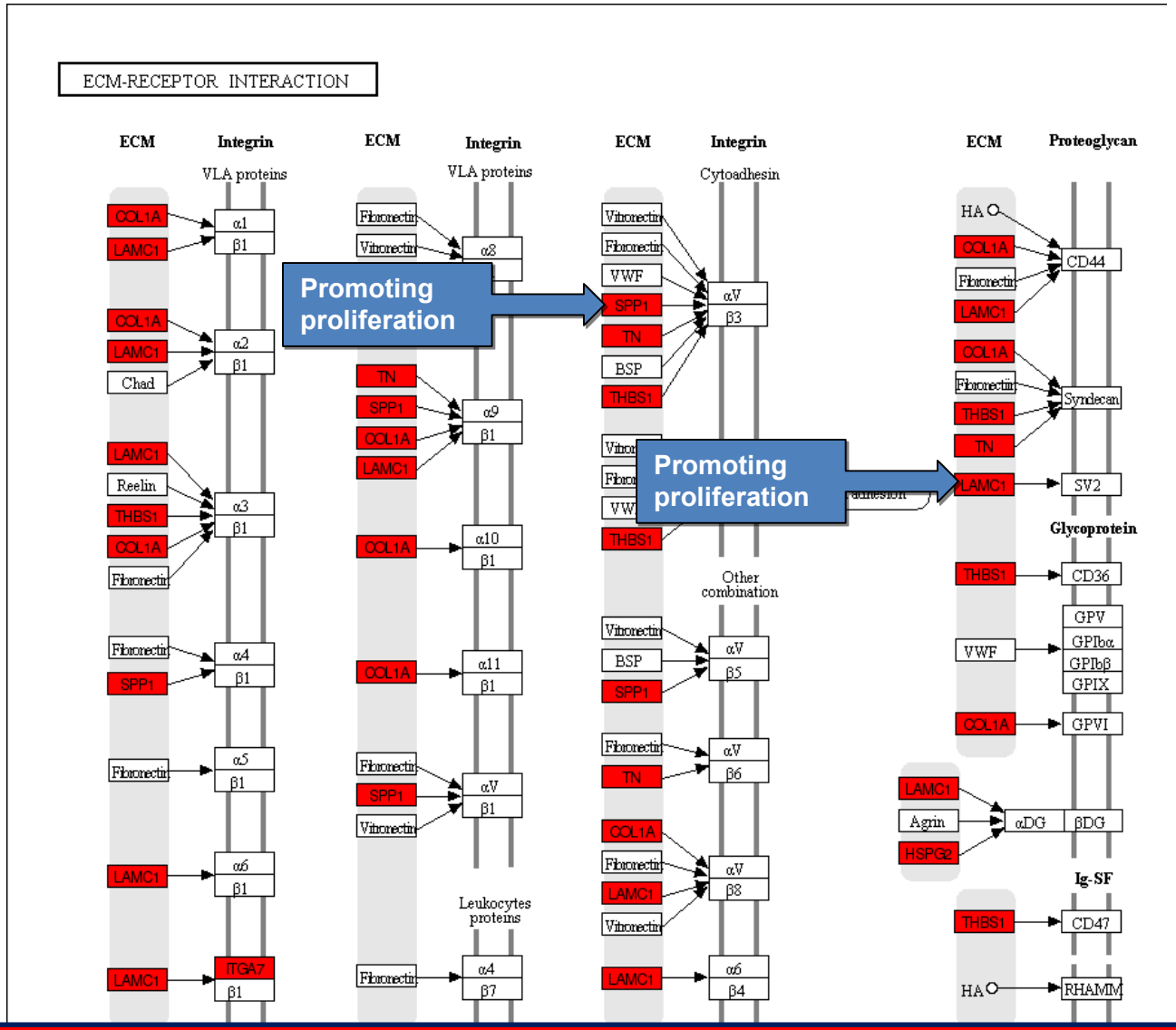
***ECM-receptor interaction*（up regulated 16 mRNAs, down regulated 0 mRNAs, q=0.007）;**

***Arachidonic acid metabolism*（ up regulated 10 mRNAs , down regulated 2 mRNAs , q=0.011）;**

***Linoleic acid metabolism*（ up regulated 5 mRNAs , down regulated 1 mRNAs , q=0.032）;**

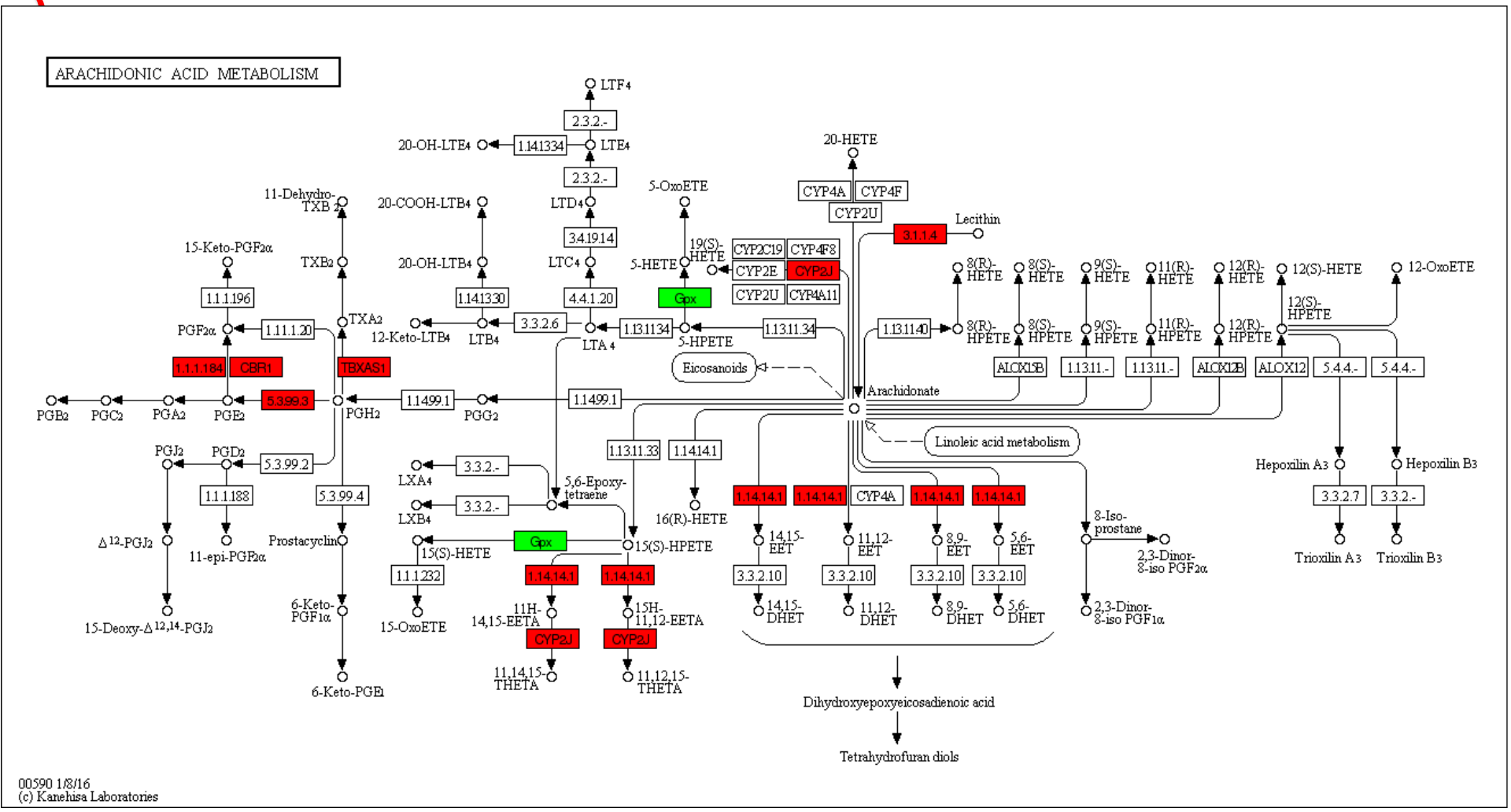
Ovarian steroidogenesis（ up regulated 4 mRNAs , down regulated 4 mRNAs , q=0.046）.

Result （V79 analysis of KEGG）



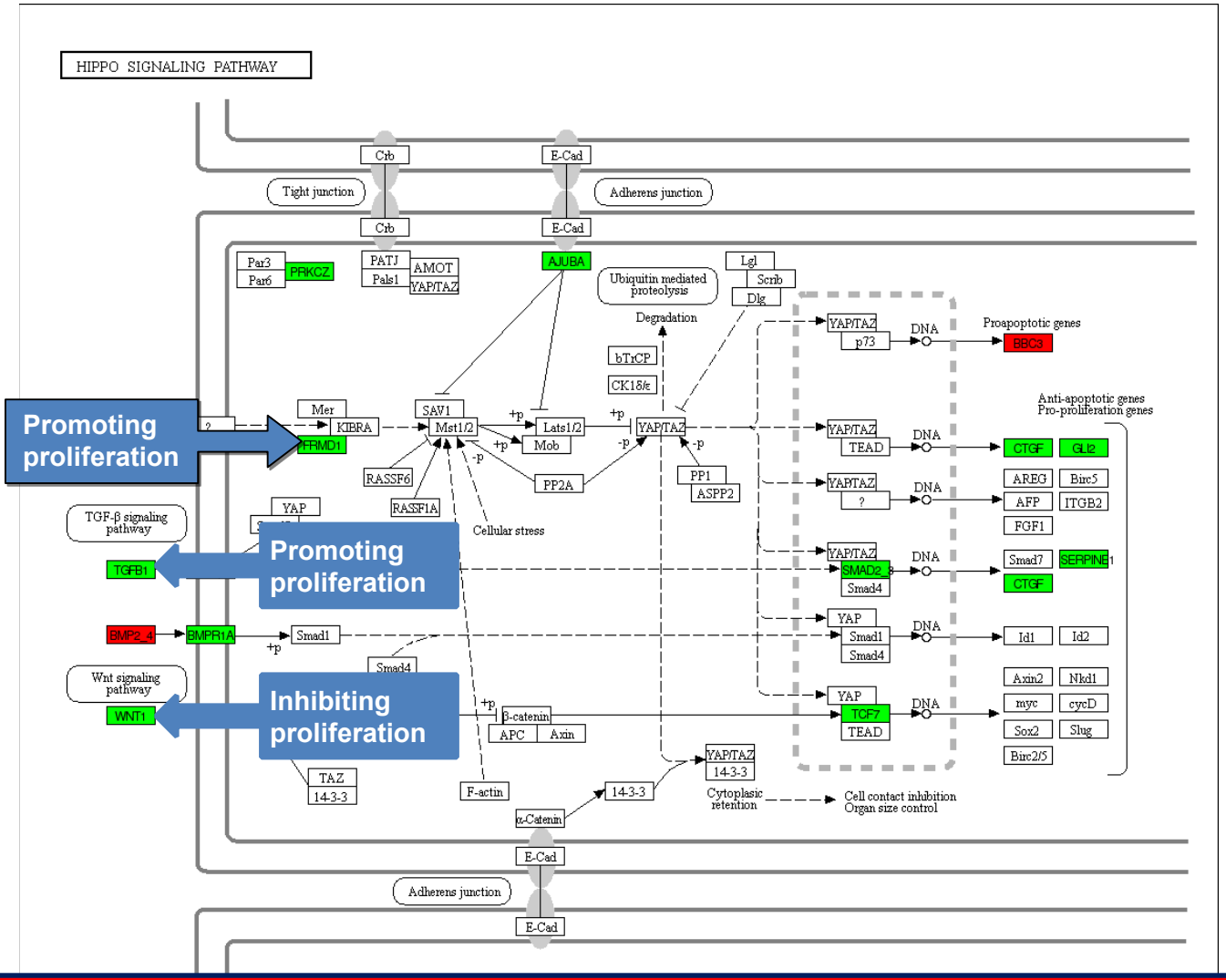
Red indicates down regulated and green indicates up regulated in Group of DUGL

Result （V79 analysis of KEGG）



Red indicates down regulated and green indicates up regulated in Group of DUGL

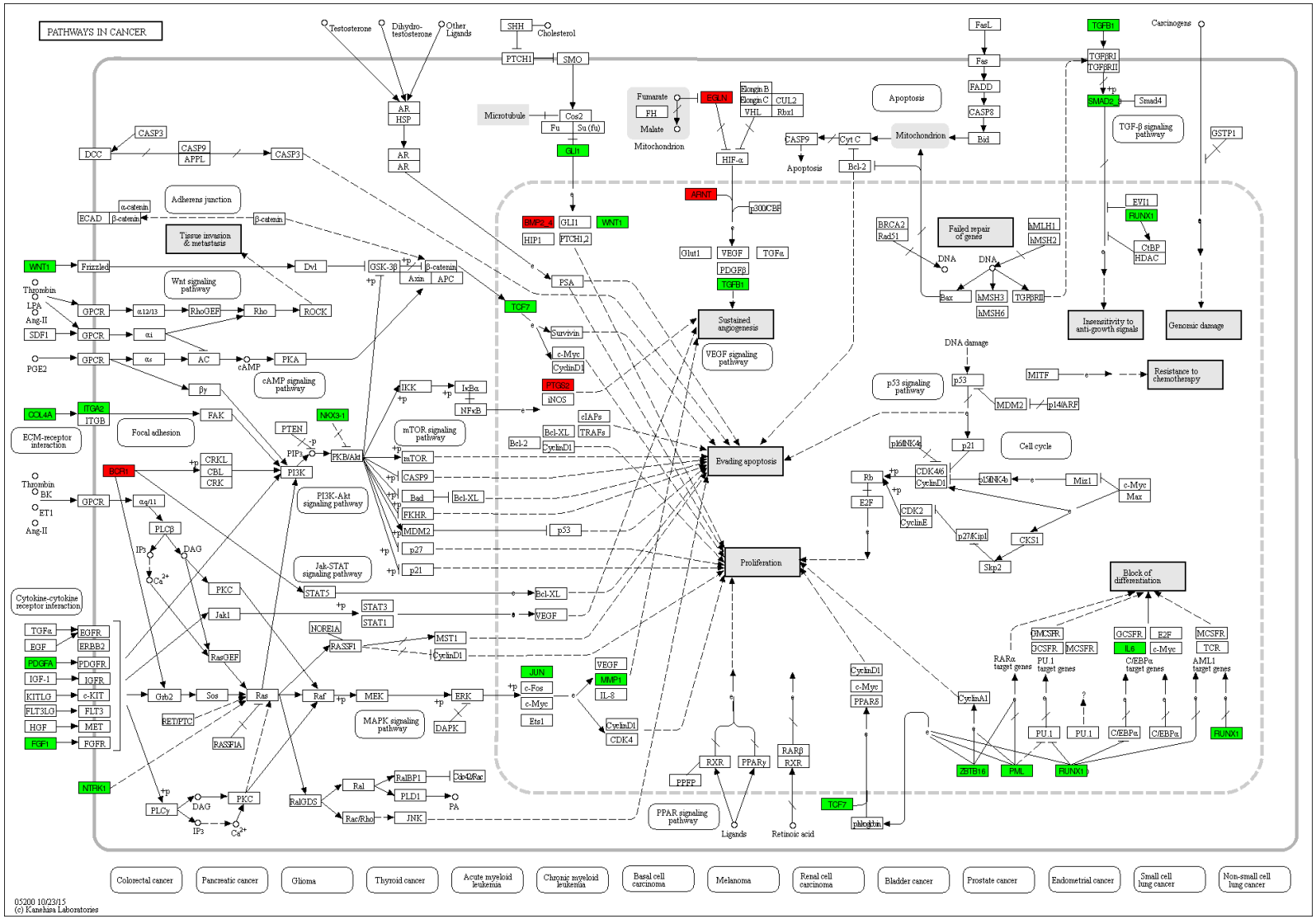
Result （KEGG analysis of FD-LSC-1）



Hippo pathway （up regulated 13 mRNAs，down regulated 3 mRNAs，q=0.014）。

Red indicates down regulated and green indicates up regulated in Group of DUGL

Result （ KEGG analysis of FD-LSC-1）



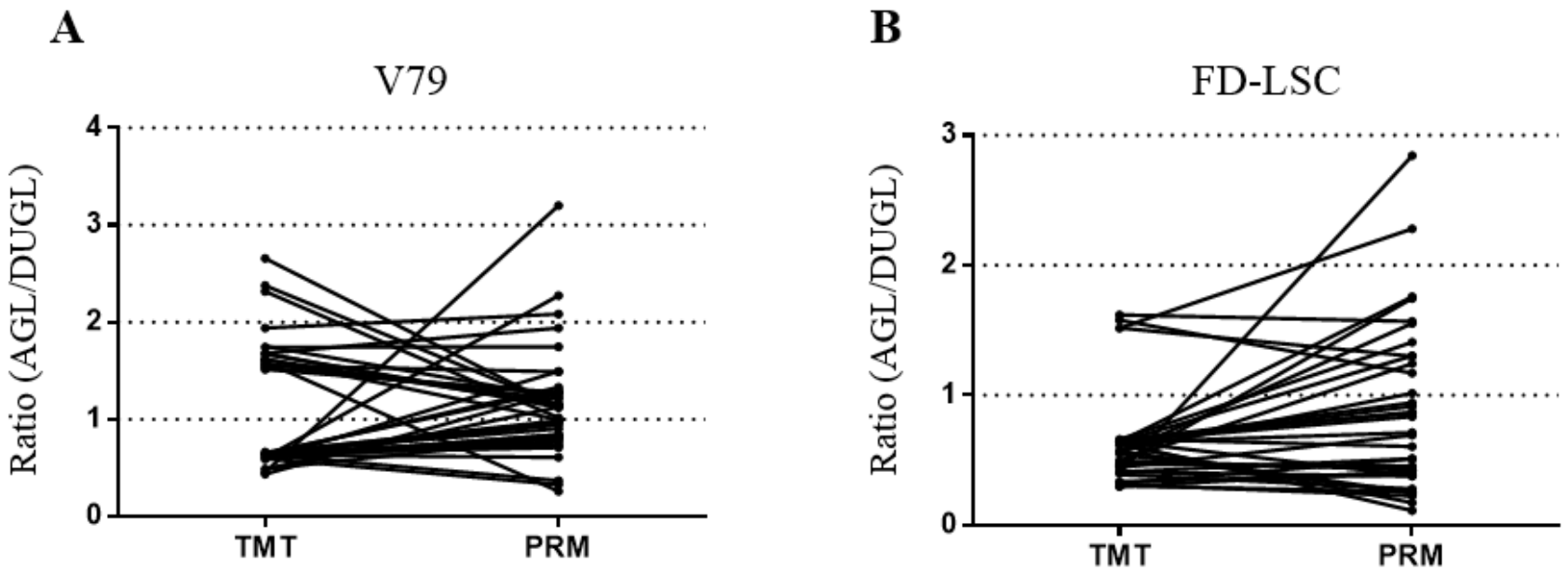
Pathway in cancer（ up regulated 17 RNAs， down regulated 7 RNAs个，*q*=0.036）

V79 and FD-LSC-1 cells Top 10 DE mRNAs(DUGL/AGL)

Gene name	Fold change	*p* value	Up/Down
Nckap5	0.0056415	7.84E-20	down
Slc28a1	0.0160969	9.86E-07	down
Nxpe2	0.0218731	0.002662613	down
Arhgap36	0.0221165	4.33E-91	down
Pacsin1	0.0230447	2.54E-05	down
Ccl20	0.0248605	4.21E-05	down
LOC100773087	0.0254093	0.000137474	down
LOC103158813	0.0257106	0.000328319	down
LOC100753743	0.0262169	3.36E-08	down
LOC103164300	0.0266047	5.18E-08	down
Ticam2	42.718612	8.34E-05	up
Adcy3	42.583997	1.50E-09	up
Cd160	35.335689	0.000540594	up
Lrch2	26.830512	0.007457903	up
Nr4a3	22.897967	1.75E-101	up
LOC100767001	21.729211	0.017595765	up
Kif27	20.435689	0.009202435	up
Mmd2	19.811003	0.037665043	up
Trnag-ccc	19.432191	0.036338422	up
LOC100752969	19.246685	0.03668432	up

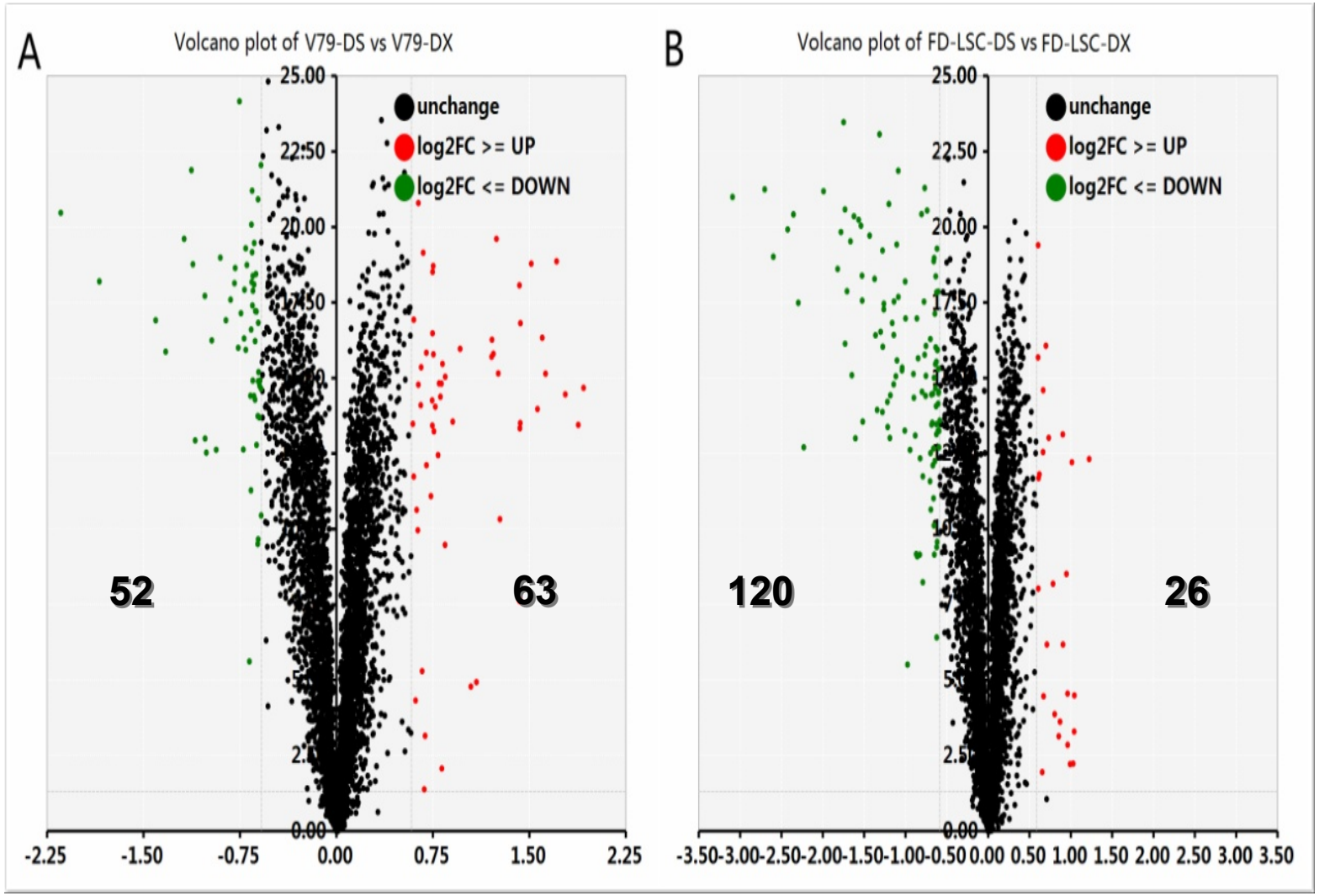
Gene name	Fold change	*p* value	Up/Down
CA9	0.0160692	6.53E-86	down
NDUFA4L2	0.0584955	0.0001835	down
ALPI	0.0760951	2.36E-150	down
ARNT2	0.1060216	1.37E-89	down
FSTL4	0.1175402	1.63E-14	down
NRG2	0.1282636	3.95E-05	down
PPFIA4	0.1404052	9.14E-07	down
ZNF658B	0.1546867	8.07E-06	down
AC090826.1	0.1552121	2.25E-13	down
PCDH12	0.1747767	0.000947	down
AC137936.2	47.384247	8.81E-05	up
AC099494.1	41.887396	0.0005322	up
MTND5P2	37.491532	0.0011268	up
TGIF2P1	33.442724	0.0033913	up
DACT1	32.413209	5.17E-10	up
SERPINE1	22.354606	1.02E-108	up
NXPH3	18.626337	0.0025685	up
ISLR2	18.239006	0.0003221	up
LBH	17.727096	1.39E-28	up
KRT4	17.026456	4.10E-08	up

Result （PRM verification of DEPs）

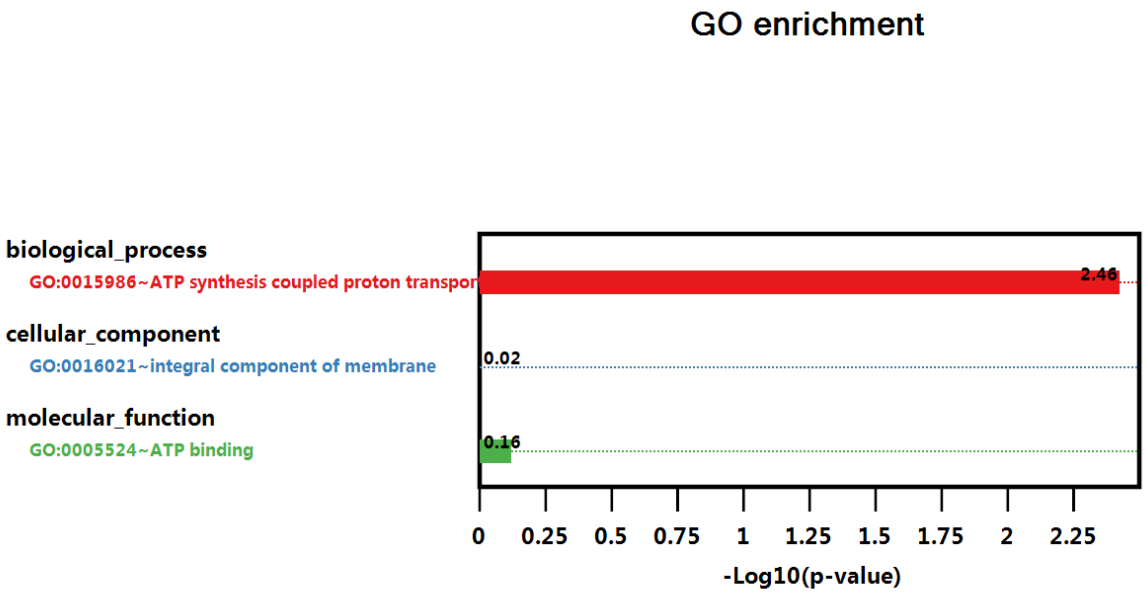


The selected DE proteins verified by PRM showed near 80% were consistent with the TMT.

Result （DE protein ）（DS=AGL，DX=DUGL）

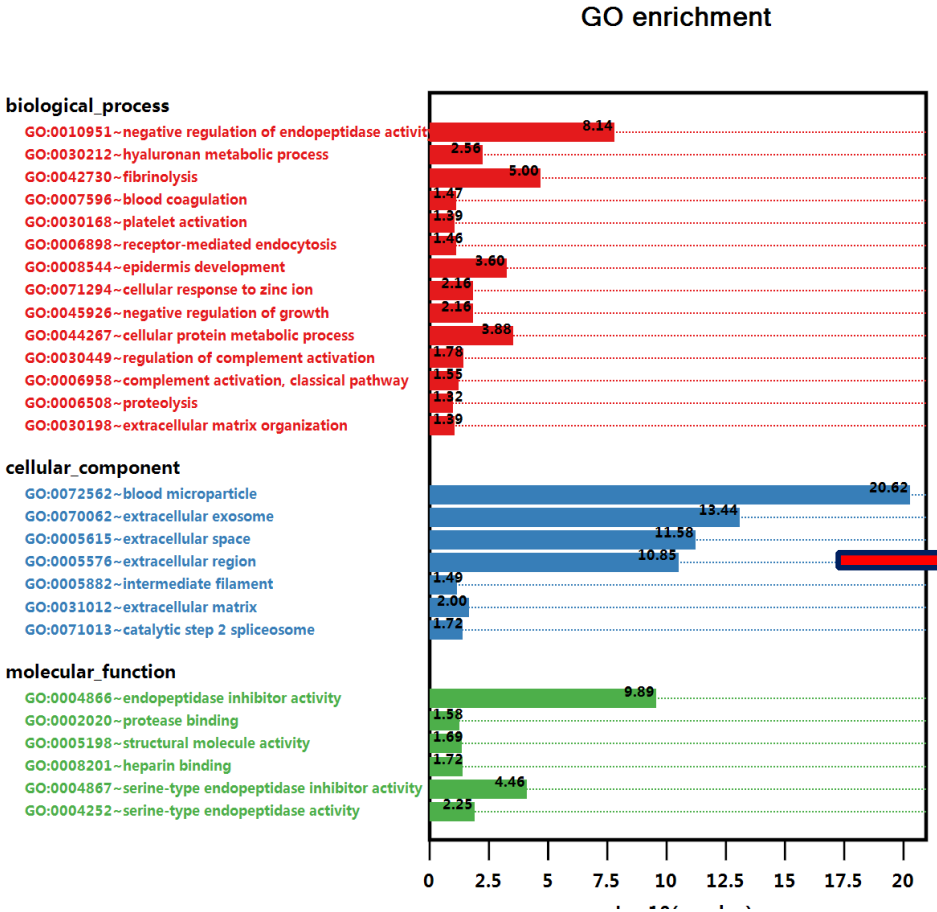


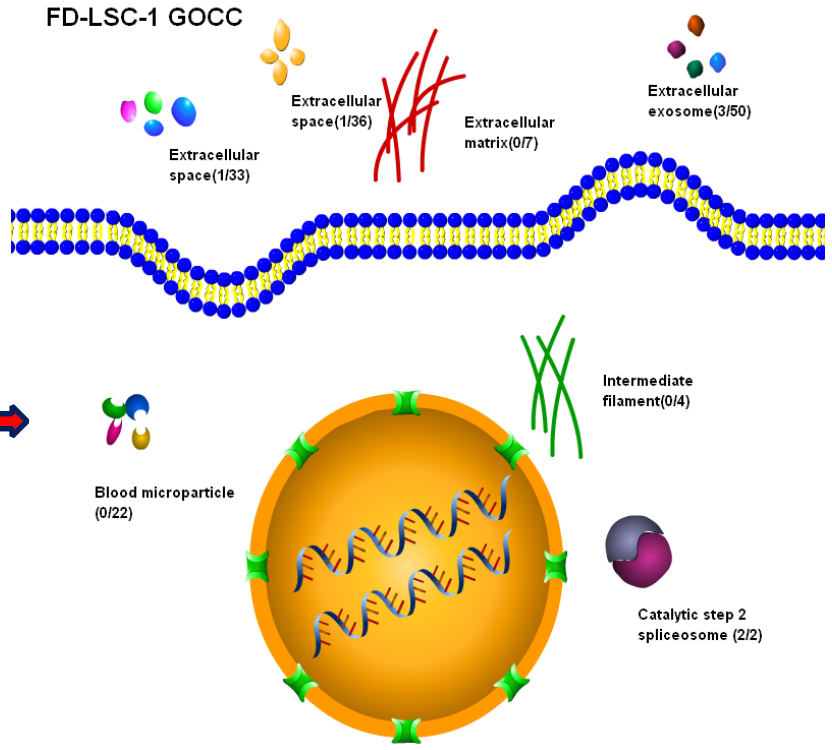
Result （V79 GO analysis of DE proteins）



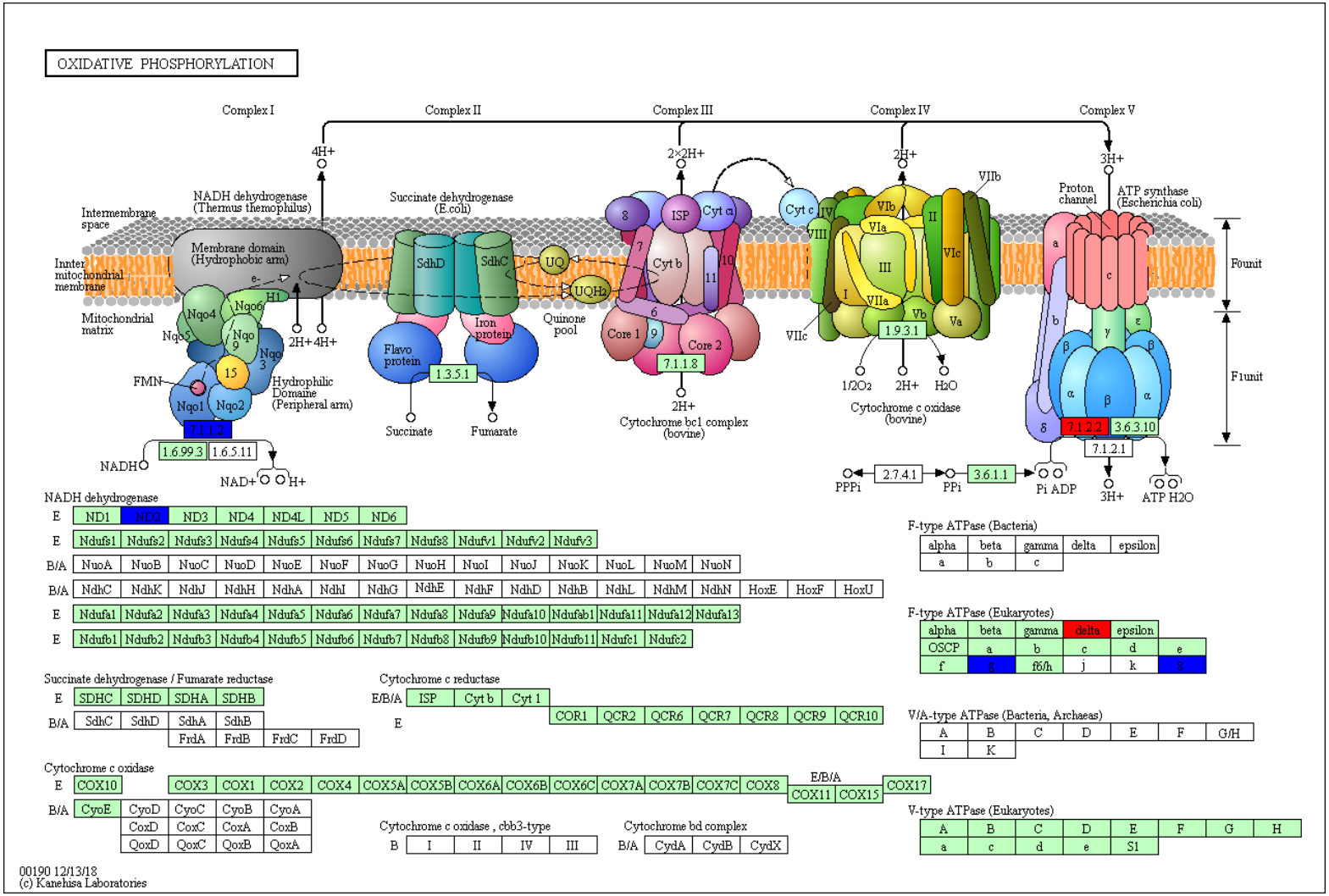
DE proteins enriched in the terms of ATP synthesis coupled proton transport of BP catalogy

Result （FD-LSC-1 GO analysis of DE proteins)



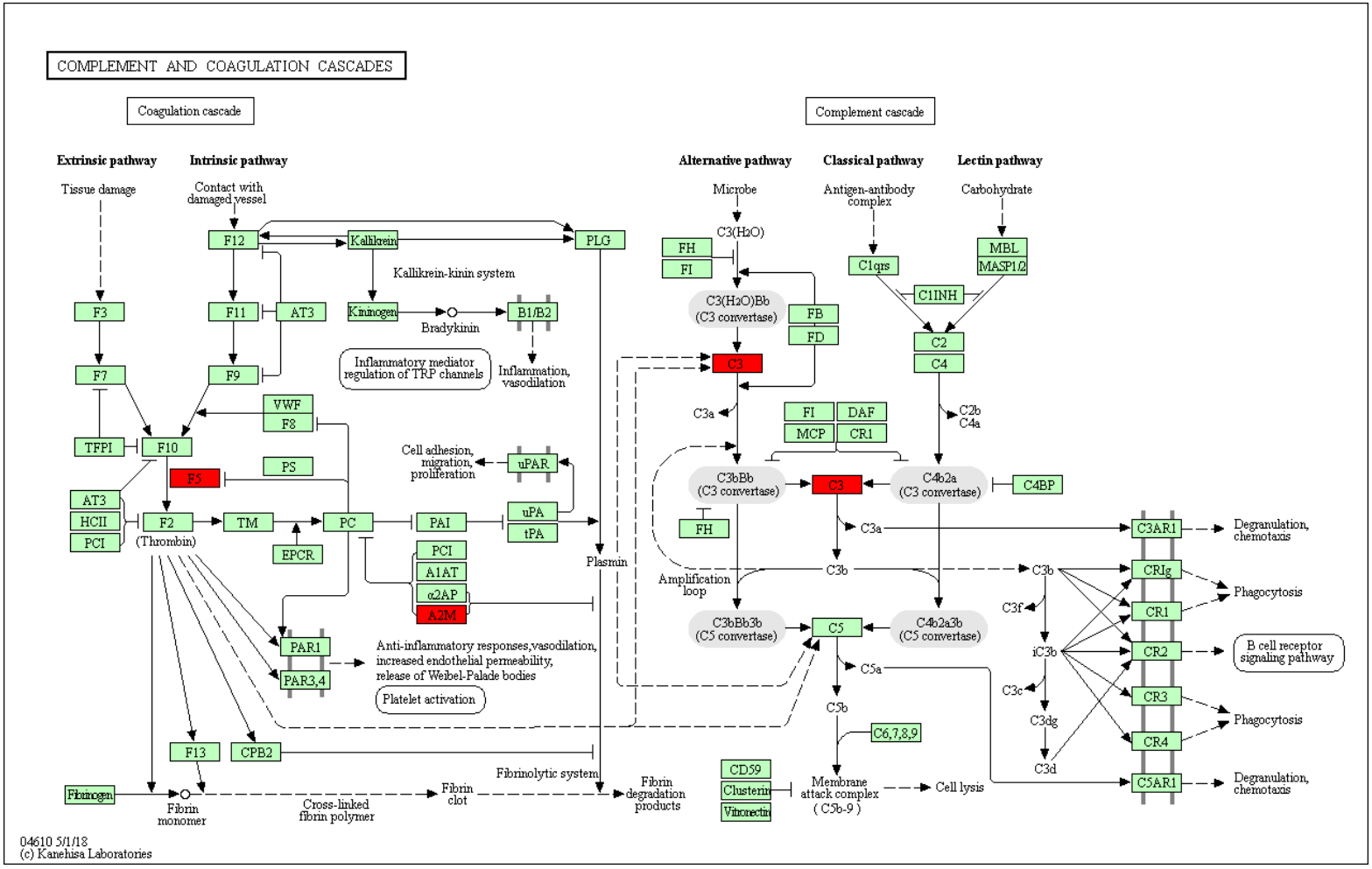


Result （V79 KEGG analysis of DE proteins）



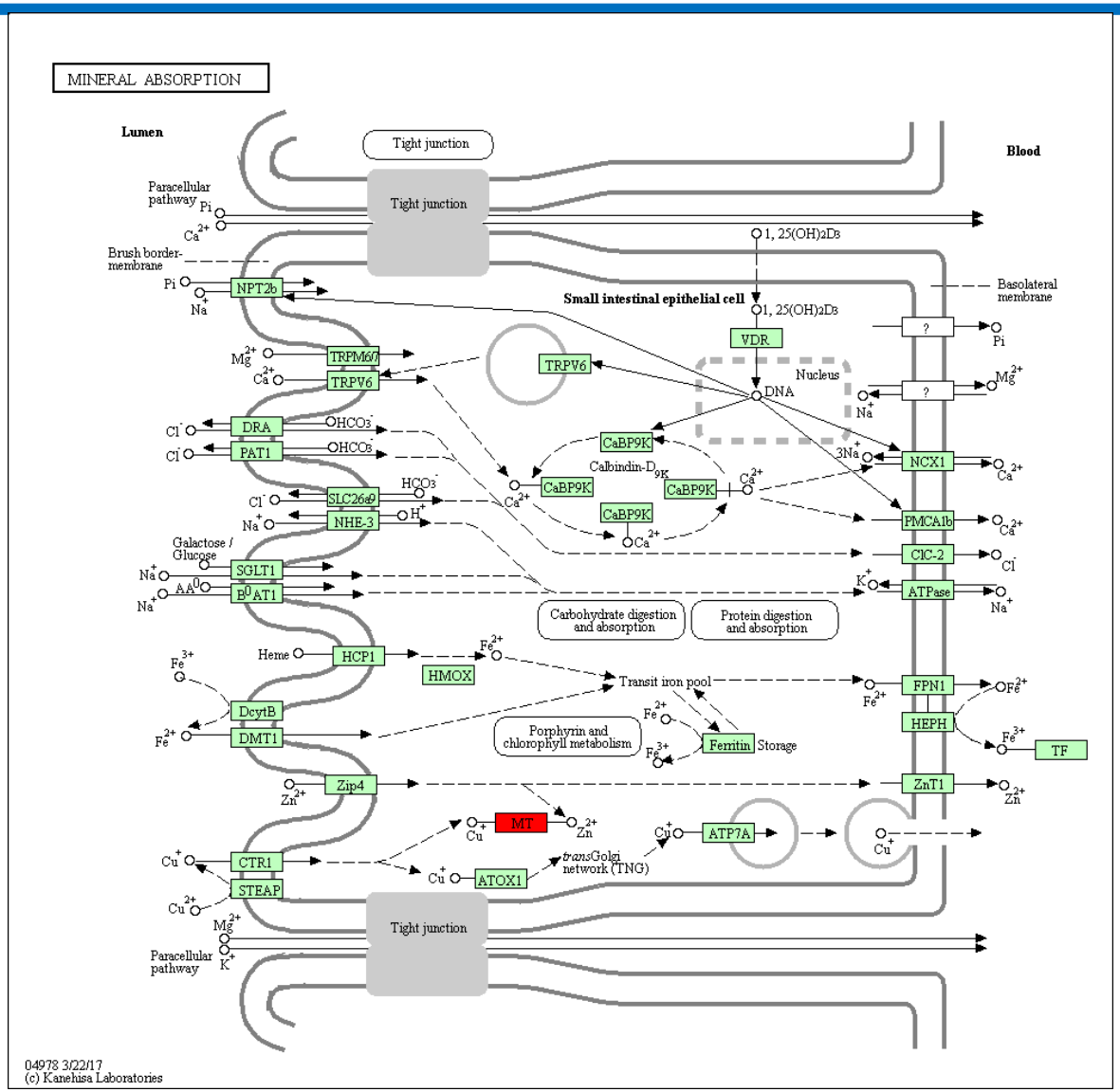
In this pathway, 1 protein un regulated and 3 down regulated in the V79 cells grow in DUGL

Result （FD-LSC-1 KEGG analysis of DE proteins）



In this pathway, 10 proteins un regulated in the FD-LSC-1 cells grow in DUGL

研究结果：蛋白组分析（FD-LSC-1 KEGG富集）



In this pathway, 3 proteins un regulated in the FD-LSC-1 cells grow in DUGL

Result(common expression of DE mRNAs and DE proteins)

Gene name	RNA fold change	Protein fold change
Rab40b	0.20327893	0.37288889
Slc14a1	0.219474162	0.435444484
Cd68	0.258149522	0.56611101
Slc1a4	0.311500279	0.634555614
LOC100772325	0.383757833	0.588888961
S100a4	0.421108129	0.51511104
Plod1	0.473107617	0.572777708
P4ha2	0.488764764	0.569444302
Ncl	2.053055049	1.632221355
Npm1	2.060942057	1.56977739
Pa2g4	2.097975454	1.50222329
Nop16	2.110555097	1.632778352
Cornifin-A	3.088526433	1.643109947
Rbm3	4.284765516	4.43577005

V79 (DUG/LAGL)

Gene name	RNA fold change	Protein fold change
SCD	0.398060807	0.538333374
ALPI	0.076095162	0.633777823
NCCRP1	0.425795546	0.658999862
GABARAPL1	2.139554588	1.53744446
C18orf25	2.050159195	1.546666007
THBS1	3.068746049	1.611889295
CYR61	2.360700939	2.005000471
FSTL1	2.042721477	2.208778126
RBM3	2.456984347	2.244109773

FD-LSC-1（DUGL/AGL）

Result(function analysis of common expression of mRNAs and proteins of V79)

14 DE mRNAs and proteins had the same expression model, 8 of them down -egulated and 6 of them up-regulated in the group of DUGL.

GO analysis shown that those DE proteins and mRNAs only involved in the terms of protein hydroxylation of BP category. As to MF, L-ascorbic acid binding, monosaccharide binding, ioxygenase activity and oxidoreductase activity were significantly enriched.

KEGG analysis shown that the pathways of Arginine and proline metabolism(P4ha2), Lysosome (Cd68) and Lysine degradation(Plod1) were significantly enriched.

Result(function of common expression of mRNAs and proteins of FD-LSC-1)

9 DE mRNAs and proteins had the same expression model,3of them down -egulated and 6 of them up-regulated in the group of DUGL.

GO analysis shown that those DE proteins and mRNAs mainly involved in the terms of binding of MF category. As to BP, positive regulation of cellular amide metabolic process and cell-substrate adhesion, response to temperature stimulus, regulation of cellular amide metabolic process , positive regulation of transmembrane receptor protein serine/threonine kinase signaling pathway and oxidoreductase activity were significantly enriched.

KEGG analysis shown that the pathways of Rap1、P13K-Akt，TGFβ，AMPK and ECM-receptor interaction et al.THBS1、ALPI and SCD enriched in many pathways。

Summary

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Discussion

- The radon concentration in DUGL of CJEM is 3.7-5.5 pCi/L, slightly higher than LNGS; the total γ radiation dose rate is 0.03-0.05μSv/h, similar with LNGS.
- The relative humidity is 99%, and with the approximately same level in LNGS.
- Except for O_2, the concentration of CO_2 and air pressure of DUGL are slightly higher than AGL.
- Light and O_2 were kept at the same level outside of the CO_2 incubator in our study.
- CO_2, relative humidity and temperature of DUGL were higher in than AGL, the three parameters were controlled inside of the CO_2 incubator.
- Pinheiro et al. show that high pressure(1.2-6 bar) could promote the proliferation of cultures. Besides, the air pressure was only a little higher in DUGL compared with AGL, the slight pressure increase might been neglected by the shift of liquid and gas. Herein, the inhibition of proliferation caused by the change of air pressure might been excluded.
- **Therefore, we suspected that the inhibition effect on the proliferation of cultures was induced by the dramatically decreased cosmic radiation.**

Discussion

- **After two days growth in DUGL,V79 cells showed a change of gene prolife with 1118 mRNA down regulated; 386mRNAs up regulated.**
- **The top up-regulated mRNAs included Adcy3、Nr4a3、Smad7、LOC103158805 and Tfrc; The top down-regulated mRNAs included Nckap5、Slc28a1、Nxpe2、Arhgap36 and Pacsin1.**
- **After four days growth in DUGL,FD-LSC-1 cells showed a change of gene prolife with 280 mRNA down regulated; 623 mRNAs up regulated. The top up-regulated mRNAs included SERPINE1、DACT1、AC137936.2、LBH and NXPH3; The top down-regulated mRNAs included CA9、NDUFA4L2、ALPI、ARNT2 and FSTL4.**

Discussion

- **DEGs mRNAs of V79 cells mainly enriched in the terms related to response to stimulus and organismal process ; terms of CC related to part of extra cell; terms of MF related to oxidoreductase activity .**
- **DEGs mRNAs of FD-LSC-1cells mainly enriched in the terms of BP related to response to external stimulus, tissue development and negative regulation of cellular process et al. CC enriched in the terms related to extra cell ; MF enriched in terms of growth factor activity and transcription regulatory .**
- **This suggested that different cells might arise different response when they face to the low background radiation stress.**

Discussion

- **DE mRNAs enriched in the pathway of ECMRI、AAM、LAM、OS.**
- **In the pathway of ECMRI，many mRNAs with the function of promoting proliferation decrease in the cells grow in DUGL, such as Colla1, Thbs2,Itga7,Lama3 and Spp1. these mRNAs decreased might contributed the proliferation of cells cultured in DUGL.**

ECM-receptor interaction	0.007	16	Hspg2, Col1a1, Thbs2, Itga2b, Itga7, Lama3, LOC100772575, LOC103158589, LOC100767397, Col4a2, LOC103161875, Spp1, LOC103164586, Col6a3, Lamb3, Col6a1 LOC100773326, LOC100764471,	0

Discussion

- **Glutathione peroxidase 3 (GPx3) had an important role in aiding cellular survival in changing environments.**
- **The GPx3 up regulated in V 79 cells of DUGL group might help to the cell survive in the deep underground environment.**
- **Besides, low background radiation might arise the change of Arachidonic acid metabolism.**

Arachidonic acid metabolism	0.011	10	LOC100765057, Ggt6, Plb1, Pla2g4e, LOC100753466, Ptges, Pla2g6, Tbxas1	2	Pla2g4c, Gpx3
Linoleic acid metabolism	0.032	5	LOC100764471, LOC100765057, Plb1, Pla2g6, Pla2g4e	1	Pla2g4c
Ovariansteroidogenesis	0.046	4	LOC100764471, LOC100765057, Pla2g4e, Bmp6	4	Hsd17b7, Pla2g4c, Adcy3, LOC100754457

Discussion

- **The Hippo signaling pathway is well known to play a central role in regulating cell proliferation through its response to mechanical stimuli. In this pathway, the mRNA with the function of inhibiting proliferation increased in the group of DUGL, such as FRMD6、WTIP、WNT9a. In contrast, the WNT6 and BMP2 with the function of promotin growth decreased.**
- **This suggested that FD-LSC-1 proliferation inhibition caused by dys-regulation of proliferation mRNAs.**

Hippo signaling pathway	0.014	3	BBC3，WNT6，BMP2	13	GLI2, TGFB2, SERPINE1, CTGF, WNT10A, BMPR1B, FRMD6, WTIP, WNT9A, TCF7L1, PRKCI, SMAD, BMPR2
Pathways in cancer	0.036	7	PTGS2, WNT6, BMP2, EGLN3, BCRP2, ARNT2, BCRP7	17	LAMC2, GLI2, MMP2, ITGA6, RARA, NT10A, IL6, TPM1, NT9A, TCF7L1, FGF18, RUNX1, SMAD3, KX3-1, JUN, PDGFA

Discussion

Oxidative phosphorylation	0.00503	3	Q27PQ4, P14414, Atp5l	1	Atp5d

- **Oxidative phosphorylation provides energy for cell growth and reproduction. Among this pathway, ATP synthase protein 8(P14414) , Mitochondrial ATP synthase g (Atp5l), NADH-ubiquinone oxidoreductase chain 2(P14414) decreased in the cells grown in DUGL.**
- **These decreased proteins might be contribute to the disorder of energy production and then inhibit the proliferation. Also, this might been reflected in the TEM scan, which shown mitochondrial swelling, and mitochondrial crista decreasing in DUGL cells.**
- **This is similar to Castillo et al.'s result.**

Discussion

FD-LSC-1				
Complement and coagulation cascades	5.82E-10	0	10	SERPINF2, PLG, C4A, SERPINC1, C9, A2M, C3, F5, F2, FGB
Mineral absorption	0.038774	0	3	MT1A, MT1E, MT2A

•Complement and coagulation cascades and mineral absorption pathway enriched in the DE proteins of FD-LSC-1. A2M and FGB with the function of inhibition proliferation increased in the group of DUGL.

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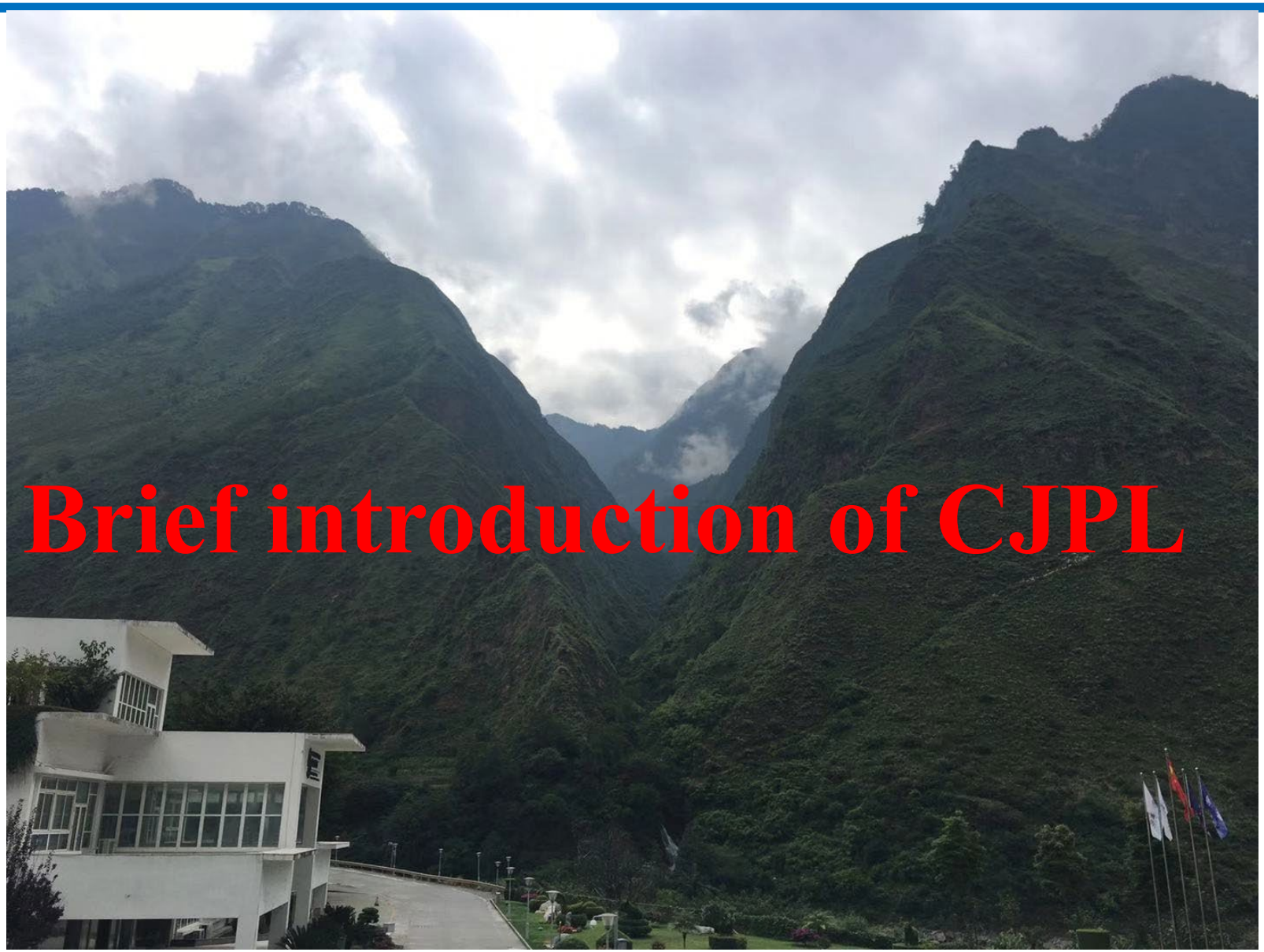
Conclusion

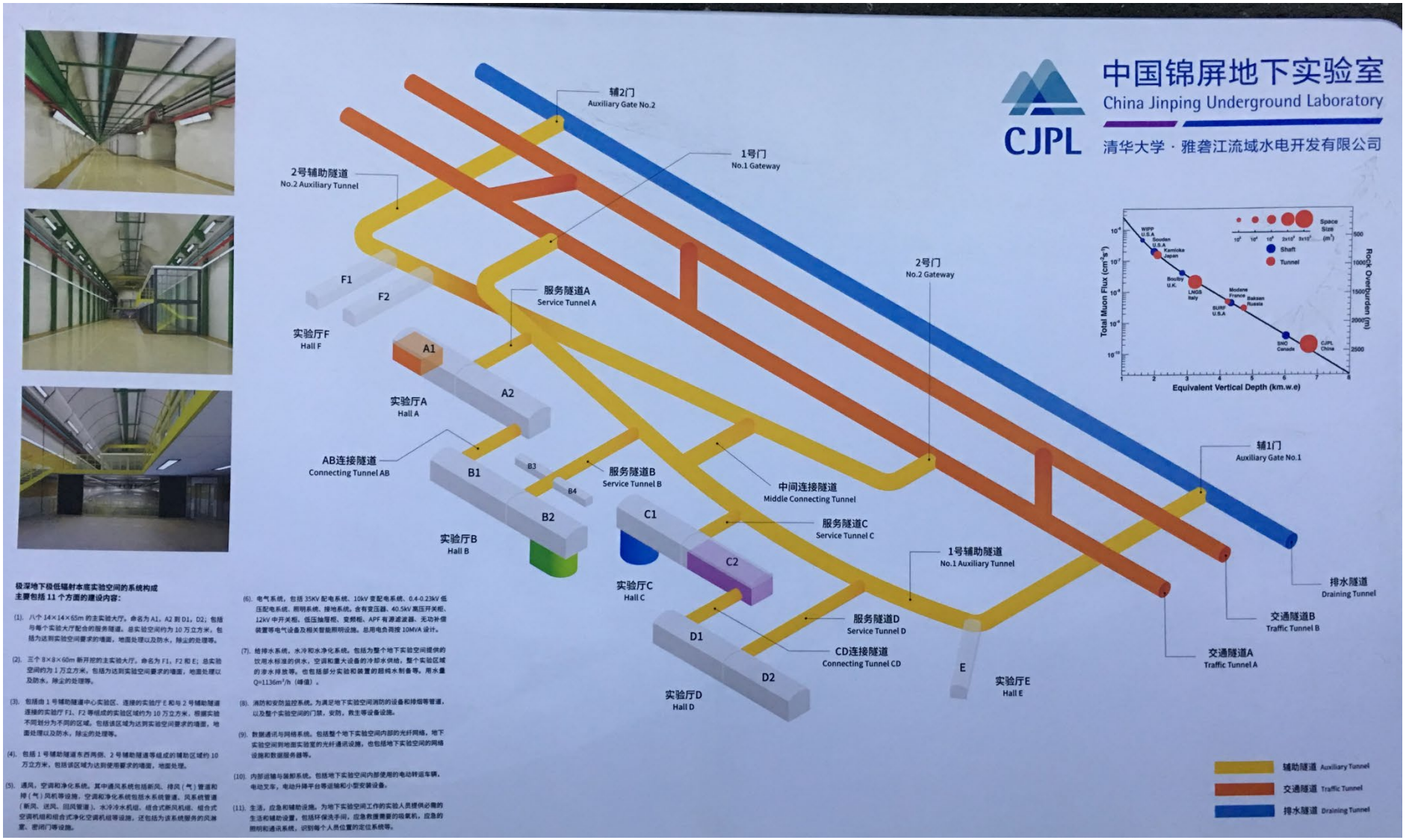
- **The environmental parameters showed that relative humidity, concertation of Rn and total γ radiation dose-rate was similar with Gran Sasso National Laboratory.**
- **And DUGL of CJEM was suitable for cells culture.**
- **According the growth curve of the cultures in AGL and DUGL, the environment of deep underground could inhibit the proliferation of cells.**
- **Among the environmental parameters measured, low background radiation might play the main role in inhibiting the proliferation of cultures in DUGL.**
- **However, the different cells might have different mechanisms in coping with low background radiation. V79 cells present the disorder of energy synthesis and oxidative metabolism in DUGL. FD-LSC-1cells growth inhibition mainly caused by the genes and protein with function of promoting cell proliferation decreased, and the genes and proteins with inhibition of proliferation increased in DUGL group.**


Brief introduction of CJPL

Brief introduction of CJPL (location)

Sichuan university to CJEM:3000 km;
Sichuan university to CJPL:400km

CJPL(schematic form)



极深地下极低辐射本底实验空间的系统构成
主要包括 11 个方面的建设内容：

(1). 八个 14×14×65m 的主实验大厅。命名为 A1、A2 到 D1、D2；包括与每个实验大厅配合的服务隧道。总实验空间约为 10 万立方米，包括为达到实验空间要求的墙面，地面处理以及防水，除尘的处理等。

(2). 三个 8×8×60m 新开挖的主实验大厅。命名为 F1、F2 和 E；总实验空间的为 1 万立方米，包括为达到实验空间要求的墙面，地面处理以及防水，除尘的处理等。

(3). 包括由 1 号辅助隧道中心实验区、连接的实验厅 E 和与 2 号辅助隧道连接的实验厅 F1、F2 等组成的实验区域约为 10 万立方米。根据实验不同划分为不同的区域。包括该区域为达到实验空间要求的墙面，地面处理以及防水，除尘的处理等。

(4). 包括 1 号辅助隧道东西两侧、2 号辅助隧道等组成的辅助区域约 10 万立方米，包括该区域为达到使用要求的墙面，地面处理。

(5). 通风，空调和净化系统。其中通风系统包括新风、排风（气）管道和排（气）风机等设施，空调和净化系统包括水系统管道、风系统管道（新风、送风、回风管道）、水冷冷水机组、组合式新风机组、组合式空调机组和组合式净化空调机组等设施，还包括为该系统服务的风淋室、密闭门等设施。

(6). 电气系统，包括 35KV 配电系统、10kV 变配电系统、0.4-0.23kV 低压配电系统、照明系统、接地系统。含有变压器、40.5kV 高压开关柜、12kV 中开关柜、低压抽屉柜、变频柜、APF 有源滤波器、无功补偿装置等电气设备及相关智能照明设施。总用电负荷按 10MVA 设计。

(7). 给排水系统，水冷和水净化系统。包括为整个地下实验空间提供的饮用水标准的供水，空调和重大设备的冷却水供给，整个实验区域的渗水排放等。也包括部分实验和装置的超纯水制备等。用水量 Q=1136m³/h（峰值）。

(8). 消防和安防监控系统。为满足地下实验空间消防的设备和排烟等管道，以及整个实验空间的门禁，安防，救生等设备设施。

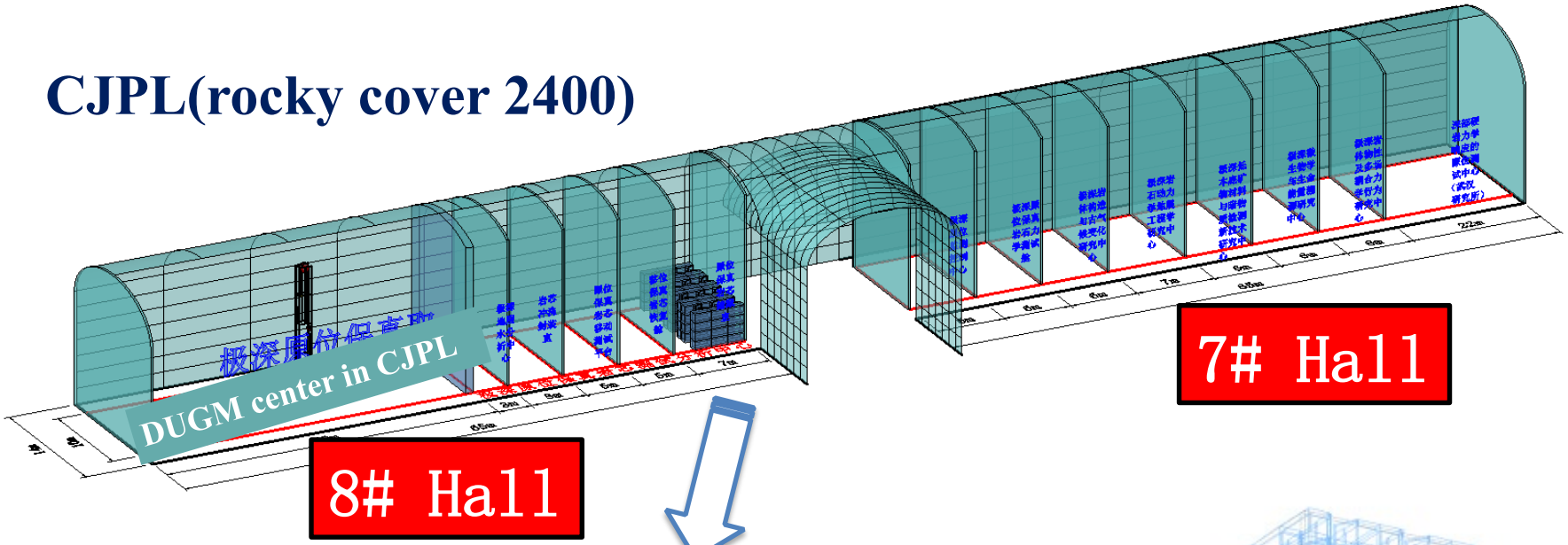
(9). 数据通讯与网络系统。包括整个地下实验空间内部的光纤网络，地下实验空间到地面实验室的光纤通讯设施，也包括地下实验空间的网络设施和数据服务器等。

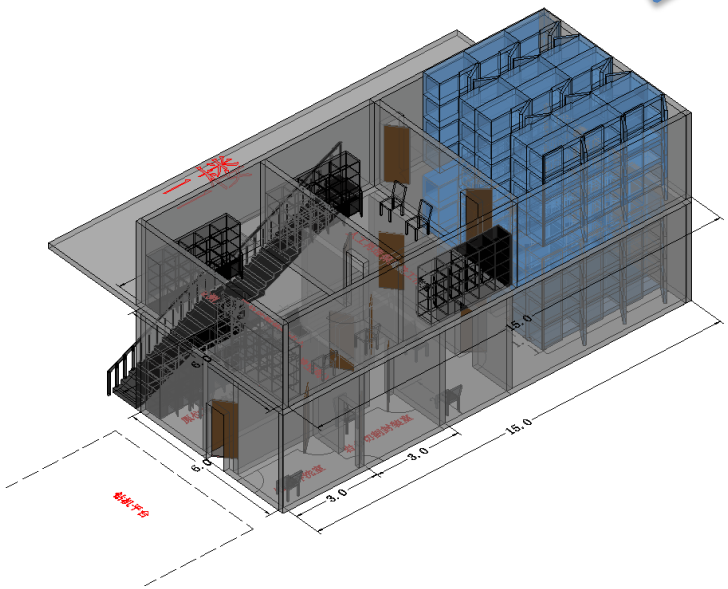
(10). 内部运输与装卸系统。包括地下实验空间内部使用的电动转运车辆，电动叉车，电动升降平台等运输和小型安装设备。

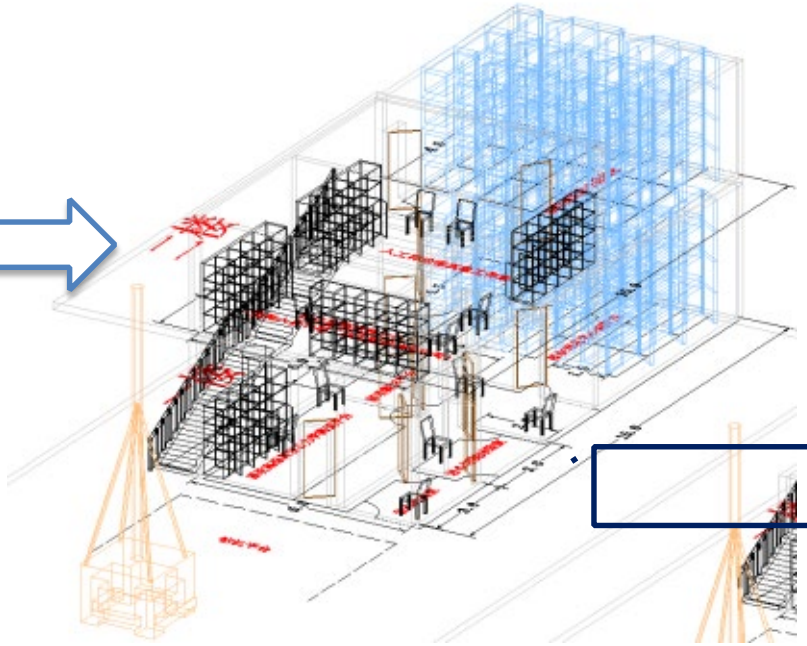
(11). 生活，应急和辅助设施。为地下实验空间工作的实验人员提供必需的生活和辅助设置，包括环保洗手间，应急救援需要的吸氧机，应急的照明和通讯系统，识别每个人员位置的定位系统等。

DUGM basis (rocky cover 2400) of CJPL

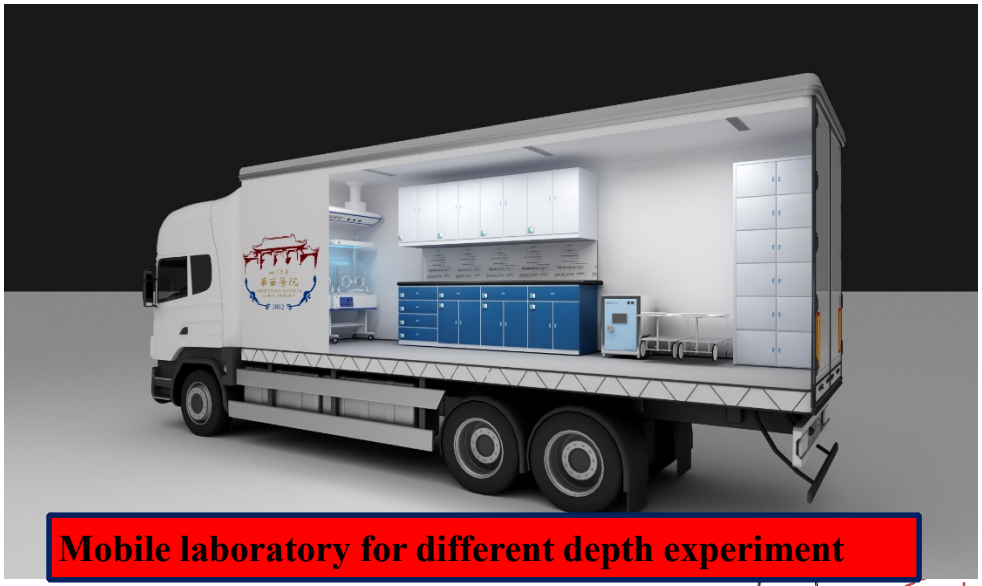
CJPL(rocky cover 2400)



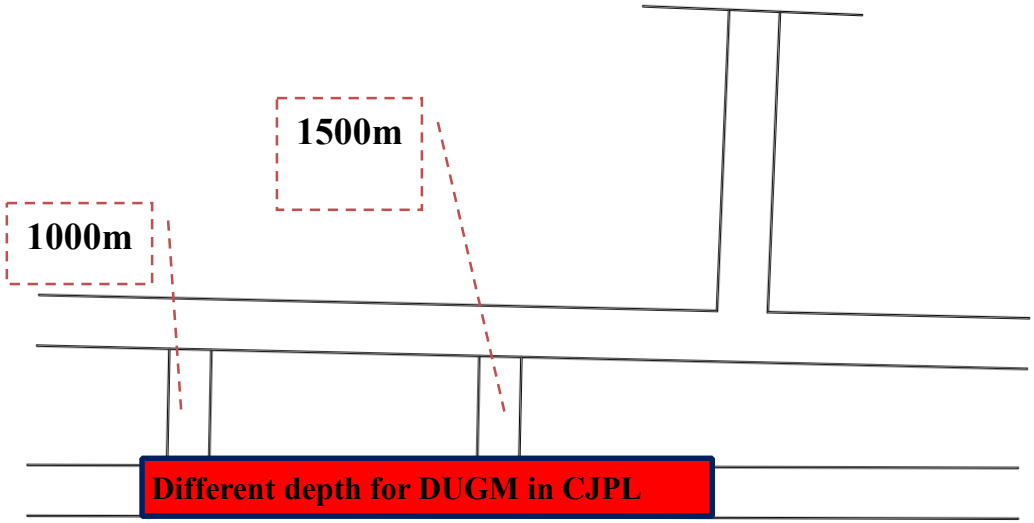




DUGM （different depth lab）basis of CJPL



Mobile laboratory for different depth experiment



Different depth for DUGM in CJPL

Above ground experiment building

Advancement of decoration of CJPL

1.3 billion ¥ for construction of CJPL phase II was supported by China central government

Acknowledge

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•We are grateful that Chairman Zhiliang Wu and his colleagues at Jiapigou Minerals Limited Corporation of China National Gold Group Corporation for their help providing an experimental site and their assistance in conducting research.
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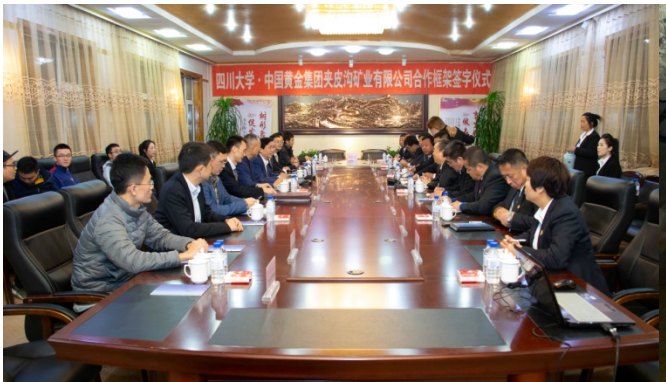

海纳百川 有容乃大

Thanks for your attention !

Welcome you to China!