in silico identification of novel cancer-related genes by comparative genomics of naked mole rat and rat

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Abstract—**The naked mole rat (NMR, *Heterocephalus glaber*) is a long-lived underground mammal, whose maximum lifespan can be up to 30 years and more than 7 times longer than house mouse. In addition, they are resistant to both spontaneous and experimentally induced tumorigenesis. These special biologic or behavioral characteristics make them most suitable for cancer and longevity research. The recent genome sequencing of NMR has provided the opportunity for the study of molecular mechanisms of such extreme traits. In this study, we carried out a comparative analysis of the complete set of NMR and rat genes. First, we identified all orthologous genes shared between these two animals. We further focused on the rat genes that were absent in NMR and used KEGG pathway database to identify the biological meaning of their proteins. The top three pathways include "Cytokine-cytokine receptor interaction", "Neuroactive ligand-receptor interaction" and "Pathways in cancer", which was consistent with the unique NMR traits. Interestingly, in the rat cancer pathway which contains 13 paths leading to evading apoptosis, 8 of them appeared to be interrupted in NMR. Finally, we found that 50% of genes lacked in "Pathways in cancer" and 40% of genes lacked in "MAPK signaling pathway" have been known to be related to a variety of cancers. Overall, this study provides insights into searching for new cancer-related genes and understanding the anti-cancer mechanism of NMR.**

Keywords-naked mole rat; cancer; PFAM family; KEGG pathway; bidirectional best hits (key words)

I. INTRODUCTION

The naked mole rat (*Heterocephalus glaber*, NMR) known as the sand puppy, is subterranean eusocial mammals native to East Africa. They have a highly unusual set of physical traits that enable them to live in the harsh, underground environment, making them one of the most extraordinary creatures known to science [1].

The maximum lifespan of NMR can be up to 30 years [2]. In comparison, a similarly sized house mouse has maximum lifespan of only 4 years, less than one seventh of NMR. The breeding females show no decline in fertility even when well into their third decade of life. Only slight age-related changes are observed in physiological characteristics [3].

In addition to delayed aging, NMRs are resistant to both spontaneous and experimentally induced cancer genesis. Cancer is a disease that generally affects most mammalian species and is usually believed to be an unavoidable accompaniment of aging. In United States, cancer leads to approximately 25% of all mortalities, making it the second leading cause of death [4]. It has been known for a long time that tumorigenesis involves a number of genes such as oncogenes and tumor suppressor genes. Identification of additional genes may help us better understand the mechanism of cancer genesis and its regulation. On the other hand, the molecular mechanisms responsible for the cancer resistance of NMR are not well understood yet. Thus, identification of NMR genes involved in cancer resistance should be important for defining causes of cancer susceptibility and resistance among mammalian species.

NMRs also have some other unique traits. Like many other poikilothermal animals, they have lost the ability to regulate their body temperature, In addition, NMRs are insensitive to pain [5] and acid [6], and possess extreme tolerance to hypoxia compared to other mammals [7], which make them well adapt to their subterranean environment.

In this paper, we carried out comparative genomics approaches [8] to investigate the genes that are either shared or specific between NMR and rat. We then focused on the rat genes that have lost in NMR. Functional analysis of these genes revealed that many of them are involved in several pathways that are related to the unique traits of NMR, such as pathways in cancer. Further analysis of the NMR-lacking genes in several cancer-related pathways revealed that many of them have been verified to be associated with different cancers. Overall, our data not only provide insights into new cancer-related genes but help understand the cancer-resistant mechanism of NMR.

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II. MATERIALS AND METHODS

A. Genome, database and resources

The complete set of annotated NMR and rat protein sequences were retrieved from NCBI (National Center for Biotechnology Information) RefSeq protein database (http://www.ncbi.nlm.nih.gov/RefSeq/). A total of 20855 and 29372 proteins corresponding to their encoding genes were obtained for NMR and rat, respectively. The KGML file of rat total pathways was obtained from KEGG PATHWAY database (http://www.genome.jp/kegg/pathway.html). The ID-mapping file of RefSeq entries, gene symbols and KEGG entries of the rat genes were obtained from UNIPROT (http://www.uniprot.org/) database.

The phenotypes of these genes were searched in the NCBI OMIM database (http://www.ncbi.nlm.nih.gov/omim) to find cancer-related genes. Furthermore, the expression data of these missing genes were searched in ArrayExpress database (http://www.ebi.ac.uk/gxa/) to identify that if these genes were differential expression in cancer compared to normal state.

B. Identification of orthologous gene pairs between NMR and rat

To analyze the orthologous gene pairs between NMR and rat, we used all annotated protein sequences of each organism as query sequences to search for homologous sequences in the other organism via BLASTP with a cutoff of E-value $\leqslant$ 1e-6. Orthologous proteins were then defined as bidirectional best hits [9].

According to the identified orthologs between NMR and rats, we divided the rat genes into three groups:

(1) Class Ⅰ: Common genes, which were present in both NMR and rat;
(2) Class II: NMR-missing genes, which were present in rat but absent in NMR;
(3) Class III: Rat-missing genes, which were present in NMR but absent in rat.

In this paper, we only focused on the first two groups of genes.

C. Gene family analysis

NMR and Rat genes in Class I was searched in PFAM database (http://pfam.sanger.ac.uk/search) to indentify the gene family each gene belonged to [10]. The number of NMR and rat of each family was calculated respectively. In order to find the family difference between NMR and rat, we used the expanded rate to sort these gene families.

D. KEGG pathway analysis

We also analyzed the pathways that Class II genes were involved in by using the KEGG PATHWAY database [11]. The KEGG pathways are consisted of nodes and their interactions. These nodes are modules composed of functionally similar genes. We mapped the genes into each pathway. If there was at least one Class II gene in a node, then the node was named "particular node". The number and percentage of particular node were calculated respectively.

III. RESULTS AND DISCUSSION

A. Distribution of orthologous genes between NMR and rat

The overlap between NMR and rat genes was shown in Fig.1. A total of 15408 genes were detected in both NMR and rat, which accounts for 73.9% of the whole NMR genes. In contrast, 5447 genes appeared to be unique in NMR. However, considering that the number of annotated NMR genes is only 70% of that of the rat genes, it is possible that some genes might be mis-annotated in the current version of NMR genome annotation.

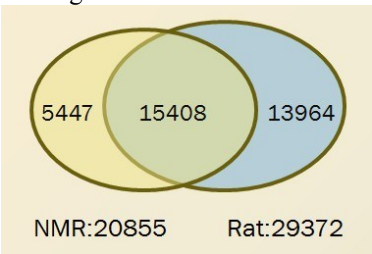

Figure 1 Venn diagram of NMR and rat genes

B. Gene family difference between NMR and rats

Proteins were divided to 13672 families by multiple sequence alignments in PFAM database. Elongation factor-2 (EF-2) family of NMR was found to expand 100% than that of rat (Table 1). It has been previously reported that eukaryotic elongation factor-2 kinase can positively modulate autophagy and negatively regulate protein synthesis to inhibit of growth factor signaling in breast cancer cells [12]. Sodium symporter (dicarboxylate symporter and solute symporter) family was another family considered to be cancer-related which resulted in tumor significantly growth delay [13].

Table 1 Gene family difference between NMR and rats*

PFAM entry	definition	Rat number	NMR number	Expanded
PF00679	Elongation factor 2 C-terminus	3	6	100.0%
PF00375	Sodium:dicarboxylate symporter	4	7	75.0%
PF01388	ARID/BRIGHT DNA binding family	7	12	71.4%
PF00571	Cystathionine-beta-synthase	9	15	66.7%
PF00128	Alpha amylase, catalytic family	3	5	66.7%
PF01661	ADP-ribose binding family	3	5	66.7%
PF02179	Bcl-2-associated athanogene	3	5	66.7%
PF00487	Fatty acid desaturase	4	6	50.0%
PF02866	lactate/malate dehydrogenase	4	6	50.0%
PF01124	Membrane-Associated Proteins	4	6	50.0%
PF00474	Sodium:solute symporter	6	9	50.0%

*only showed top 10 gene families

C. Functional analysis of genes that were absent in NMR

The top ten pathways that had the most NMR-missing genes were shown in Table 2. First, at least 31 genes had lost in the pathway of "Neuroactive ligand-receptor interaction" [14], which were consistent with the pain-insensitive trait of NMR. Second, five of these pathways were thought to be

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Table 2: The number of NMR missing genes in each pathway *

ranking	pathway name	gene number	percentage
1st	Cytokine-cytokine receptor interaction	31	5.92%
2nd	Neuroactive ligand-receptor interaction	31	5.92%
3rd	Pathways in cancer	30	5.73%
4th	Oxidative phosphorylation	26	4.96%
5th	MAPK signaling pathway	20	3.82%
6th	Ribosome	18	3.44%
7th	Purine metabolism	18	3.44%
8th	Wnt signaling pathway	17	3.24%
9th	Alzheimer's disease	15	2.86%
10th	Regulation of actin cytoskeleton	15	2.86%

*only showed top 10 pathways

Table 3: The percentage of particular node in each pathway

ranking	pathway name	special node	total node	percentage
1st	Steroid hormone biosynthesis	43	107	40.2%
2nd	Neuroactive ligand-receptor interaction	31	91	34.1%
3rd	Purine metabolism	49	153	32.0%
4th	Pyrimidine metabolism	28	88	31.8%
5th	Hematopoietic cell lineage	33	139	23.7%
6th	Pathways in cancer	46	216	21.3%
7th	Endocytosis	17	95	17.9%
8th	MAPK signaling pathway	22	125	17.6%
9th	Oxidative phosphorylation	32	191	16.8%
10th	ECM-receptor interaction	21	130	16.2%

*only showed top 10 pathways

cancer-related: “Cytokine-cytokine receptor interaction” [15], “Pathways in cancer”, “MAPK signaling pathway” [16], “Purine metabolism” [17], “Wnt signaling pathway” [18], suggesting that the absence of some of these genes might be involved in cancer resistance of NMR. The loss of 26 genes in “Oxidative phosphorylation” pathway implied that NMR may need less ATP than rat. In addition, it is well known that oxidative phosphorylation produces reactive oxygen species such as superoxide and hydrogen peroxide, which lead to propagation of free radicals, damaging cells and contributing to disease such as cancer and aging, the absence of these genes may be also related to cancer resistance and longevity.

We further calculated the percentage of NMR-missing genes in each pathway (Table 3). We found that “Steroid hormone biosynthesis” ranked first in percentage of 40.2% particular nodes. Steroid hormone consists of adrenal cortical hormone and sex hormone. So this pathway was thought to be related to NMRs’ strong reproductive capacity which shows no decline in fertility even when well into their third decade of life [19]. “Neuroactive ligand-receptor interaction” ranked second in the ratio of 34.1%. And “pathways in cancer” possessed 21.3% particular node, which ranked sixth in table 3.

D. Analysis of cancer-related genes that were absent in NMR

The main part of the cancer pathway map was shown in fig.2. The key process of cancer is “Evading apoptosis”. Interestingly, there are 13 paths leading to evade apoptosis in cancer pathway, as showed in blue or yellow lines in fig.2. It was found that 7 paths had changed by particular nodes directly related to evade apoptosis and 1 path had changed by indirect nodes. As we said before, the NMR missing genes actually mediated in these red nodes. It was found that each node contained only one gene. If the only one gene had lost in the path, then the path would actually be blocked. Genes of Survivin, Mtor, p53, Bcl-XL and Bcl-2 which can lead to evading apoptosis in cells had lost in NMR. We assumed that these 5 genes lost in NMR somatic cells may block the path to evade apoptosis, then cause cancer cells death. To explain cancer-resistance of the NMR, a two-tier protective mechanism involving contact inhibition mediated by $p16^{Ink4a}$ and $p27^{Kip1}$ was proposed [20]. But rats showed only contact inhibition mediated by $p27^{Kip1}$. Rat $p16^{Ink4a}$ had lost orthologous gene in NMR while $p27^{Kip1}$ had orthologous gene in NMR. We inferred that NMR so called “$p16^{Ink4a}$ gene” had great difference with rats.

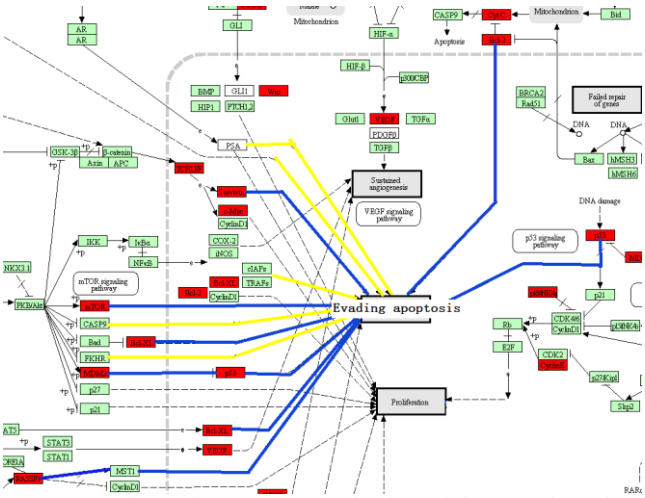

Figure 2 pathways in cancer. Red nodes denoted the particular nodes and green nodes denoted the similar nodes. Blue lines denoted the different paths; yellow lines denoted the similar paths.

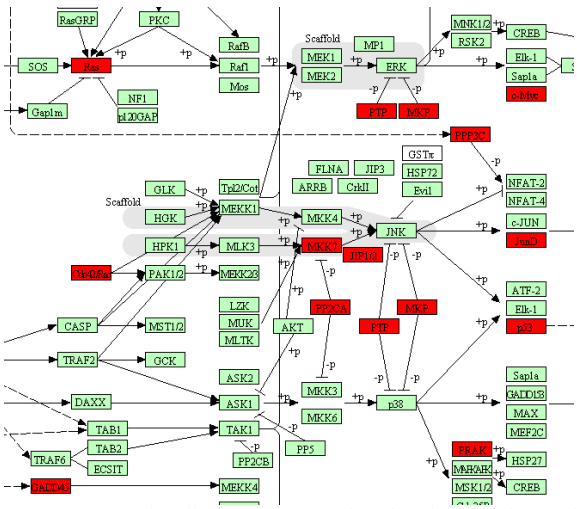

Figure 3 MAKP signaling pathway. Red nodes denoted the particular nodes and green nodes denoted the similar nodes.

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Table 4 NMR missing gene in cancer pathway

Gene Symbol	OMIM phenotype of cancer	ArrayExpress in cancer
Mdm2	Accelerated tumor formation	no
Tp53	Adrenal cortical carcinoma,etc	overexpressed
Myc	Burkitt lymphoma	overexperessed
Pdgfb	Giant-cell fibroblastoma	no
Casp8	Hepatocellular carcinoma,	overexpressed
Rara	Leukemia, acute promyelocytic	underexpressed
Bcl2	Leukemia/lymphoma	underexpressed
Rassf1	Lung cancer	no
Sufu	Medulloblastoma	no
Cdkn2a	Pancreatic cancer,Orolaryngeal cancer	overexpressed
Lef1	Sebaceous tumors	overexperessed
Fas	Squamous cell carcinoma	no
Hras1	Thyroid carcinoma	overexperessed
Fgf23	tumor-induced Osteomalacia	overexperessed
Ccdc6	Thyroid papillary carcinoma	no
Birc5	no	no
RSA14	no	no
Mmp1a	no	no
Wnt10a	no	no
Shh	no	overexperessed
Bcl2l1	no	overexpressed
Cyct	no	overexpressed
Egln3	no	overexpressed
Tceb2	no	overexpressed
Ccne2	no	overexpressed
Rac1	no	overexpressed
Figf	no	underexperessed
Igf1	no	underexpressed
Mtor	no	underexpressed
Ralb	no	underexpressed

* "no" in second column means that the phenotype wasn't cancer or there was no phenotype of the gene in OMIM. "no" in third column meant that there were no express data of the gene.

Thirty genes in the rat "Pathways in Cancer" were not found in NMR (Table 4). We found that 15 genes, which occupied 50% of the total genes, possessed the phenotype of cancer. Furthermore, among these genes, Myc, Hras1 and Pdgfb are proto-oncogene. The proto-oncogene was actually normal gene that could become the oncogene due to mutations or overexpression,thus these proto-oncogene lost in somatic cells could strengthen resistance to cancer. It was suggested that the loss of cancer-related genes. And 20 genes (66.7% of the total genes) were found to have differential expression levels in cancer compared to normal state. These genes may be related to cancer.

In the MAKP signaling pathway, the important proto-oncogene Ras had lost. As shown in fig. 3, gene Ras was the hub gene in this pathway, which was the target gene of 7 other genes. It has been reported that if NMR cells were transfected with oncogene Ras^{G12V}, cells rapidly entered crisis as the presence of anaphase bridges, giant cells with enlarged nuclei, multinucleated cells, and cells with large number of chromosomes [21]. This response permitted cells to avoid malignant tumor growth. The absence of this gene may play an important role in NMR cancer-resistance trait.

Table 5 NMR missing genes in MAKP pathway*

Gene Symbol	OMIM phenotype of cancer	ArrayExpress in cancer
Tp53	Adrenal cortical carcinoma	overexpressed
Myc	Burkitt lymphoma	overexperessed
Ptpn5	Colorectal cancer	no
Fas	Squamous cell carcinoma	no
Dusp9	Squamous cell carcinoma	overexpressed
Hras1	Thyroid carcinoma	overexpressed
Pdgfb	Giant-cell fibroblastoma	no
Fgf23	tumor-induced Osteomalacia	overexperessed
Cblp	no	no
Rac1	no	underexpressed
Pla2g10	no	overexpressed
Cacng8	no	no
Ntf4	no	no
Ppm1a	no	underexpressed
Gadd45a	no	underexpressed
Tnfa	no	underexpressed
Mapkapk5	no	overexpressed
Mapk8ip2	no	no
Map2k7	no	no
Jund	no	no

* "no" in second column means that the phenotype wasn't cancer or that there was no phenotype of the gene in OMIM. "no" in third column meant that there were no express data of the gene.

Twenty genes were found absent in "MAPK signaling pathway" of NMR (table 5). Using the OMIM database and ArrayExpress database, we showed that 40% of the genes in "MAPK signaling pathway" possessed the phenotype of cancer and 55% of the genes exposed differential expression in cancer compared to normal state. The 3 proto-oncogene: Myc, Hras1 and Pdgfb were also present in this pathway. We infered that this pathway had contributed a lot to resistance of cancer in NMR.

Many important genes, such as Wnt, Rac1, Lef1, Myc and Rhoa, were absent in the Wnt signaling pathway of NMR (fig. 4).Myc was proto-oncogene which could develop into cancer by mutation or overexpression. Rhoa has been widely confirmed as a cancer-mediated gene, which controls spreading of tumor cells [22], acts as a mediator of clinically relevant androgen action in prostate cancer cells [23], and triggers a specific signaling pathway that generates transforming microvesicles in cancer cells [24]. Lef1 interacted with a lot of other genes, such as Ctbp, Nlk, and beta-catein. These interactions were considered to be responsible for prostate cancer growth and invasion [25]. Rac1 was associated with DNA transcription. It has been reported that activation of Rac1 mediates Twist1-induced cancer cell migration [26].

Seventeen genes were found absent in "Wnt signaling pathway" of NMR (Table 6). Searching from the OMIM database and ArrayExpress database, we indentified that 5 (29.4% of the total genes) genes in "Wnt signaling pathway" possessed the phenotype of cancer and 12 (70.6% of the total genes) genes were found to have differential expression in cancer compared to normal state. Some genes, such as Ccnd3,Sox17 and Dkk4, were found to have no OMIM phenotype and differential expression so far.

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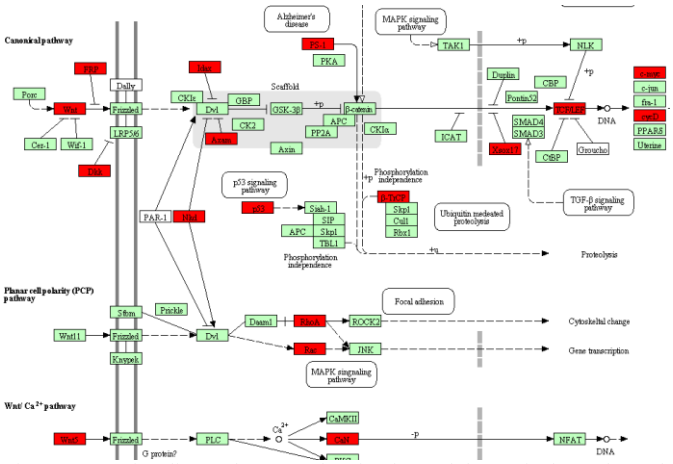

Figure 4 Wnt signaling pathway. Red nodes denoted the particular nodes and green nodes denoted the similar nodes.

Table 6 NMR missing gene in Wnt signaling pathway

Gene Symbol	OMIM phenotype of cancer	ArrayExpress in cancer
Lef1	Sebaceous tumors	overexperessed
Rhoa	Prostate Cancer	overexpressed
Rac1	Colorectal cancer	overexpressed
Myc	Burkitt lymphoma	overexperessed
Tp53	Adrenal cortical carcinoma	overexpressed
Ccnd3	no	no
Psen1	no	overexpressed
Ppp3r2	no	underexpressed
Fbxw11	no	underexpressed
Nkd2	no	underexpressed
Sfrp5	no	underexpressed
Sox17	no	no
Wnt10a	no	no
Dkk4	no	no
Wnt5a	no	overexpressed
Senp2	no	no
Cxxc4	no	underexpressed

IV. CONCLUSION

In this paper, orthologous genes were identified by the comparative genomics of NMR and rat. Then we divided the genes of these two species into three classes. Class I genes was analyzed by PFAM family database. Elongation factor-2 was found to be the most disparate gene family between NMR and rat. Class II genes were mapped to the pathway according to KEGG PATHWAY database. Five cancer-related pathways were found in the top 10 pathways descending sorted by particular node rate. Then we focused on the cancer-related pathways. It was found that 8/13 of paths to evade apoptosis had changed in cancer pathway of NMR. The important gene Ras which interacted with many other genes was absent in MAKP pathway. Five genes (Wnt, Rac1, Lef1, Myc, Rhoa), were absent in the Wnt signaling pathway of NMR In a word, these results can provide major insights into the molecular mechanisms of cancer resistance.

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