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Genomic copy number alteration of glycolytic pathway in endometrial cancer

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Summary

Metabolic reprogramming is one of the hallmarks of cancer cells, but very little is known about the difference in the expression of metabolic genes between cancer and normal tissues. The degree to which different cancer types display similar metabolic alteration is poorly understood. The best-known example of metabolic disturbance in cancer cells is the Warburg effect. The Warburg effect is the phenomenon of the cancer cells favoring the anaerobic glycolysis even in the presence of oxygen. It is displayed by most cancer cells. Although the genomic, transcriptomic, and proteomic studies have been published, metalobomic differences are still a major gap in our knowledge. Among women the most common malignancy is endometrial cancer. In this retrospective study, the authors investigated the genomic instability of glycolytic genes in endometrial carcinoma patients using array-based comparative genomic hybridization (aCGH) reports. The results indicate that among 54 patients diagnosed with endometrial carcinoma, in 21 patients, pathogenic genomic instability was detected which are linked with the disease. Among the 21 patients who had genomic instability, 19 of them (90.5%) displayed copy number variations of at least one or more glycolysis genes based on the genomic laboratory reports of the patients.

Key words: Copy number variation; Copy number alteration; Endometrial cancer; Glycolytic pathway; Metabolism; Comparative genomic hybridization (aCGH); Genomic instability.

Introduction

Cancer is a cellular disturbance with complex molecular interactions. Cancer hallmarks are categorized as: 1) strengthening the proliferative signaling, 2) unsuppressing the molecular inhibitions of cellular growth, 3) activating invasion and metastasis, 4) switching to replicative immortality, 5) increasing angiogenesis, and 6) suppressing programmed cell death [1]. Although cancer cells must have replication potential to become macroscopic tumors, it is known that genomic replication is also perturbed. Replication stress is a broad terminology and can be described as a failure of an efficient DNA replication and may lead to genomic instability which is the recurrent patterns in cancer [2].

The effect of genomic instability on tumor metabolism has begun to attract interest, and the missing links between tumor biology and genetics and metabolism have begun to be sought. The Warburg effect is one of the oldest concepts of cancer. This phenomenon is based on the experiments of Otto Warburg in the 1920s when he observed a shift from oxidative to fermentative metabolism as a common physiological feature of cancer cells [3]. Warburg used an animal model to study the rapidly growing cancer cells and compared them with normal cells; later, he developed a hypothesis that the source of cancer is a metabolism-based disorder. For a long time, the Warburg effect was not accepted. Despite the early insight into cancer metabolism, the Warburg effect is still a phenomenon. To find missing links between tumor biology including genetics and metabolism, it is necessary to analyze the cancer genome, while considering the general metabolic pathways [4]. In this retrospective study, the authors analyzed the copy number variations of glycolytic metabolic pathways in endometrial cancer patients using the genomic laboratory report of the patients including comparative genomic hybridization array (aCGH) data. The analysis of aCGH of the metabolic enzymes in endometrial cancer patients indicates that >90% of the patients have copy number variations of glycolytic genes among the patients who have genomic aberrations within their genomes based on their genomic laboratory reports.

Materials and Methods

Microarray-based comparative genomic hybridization results of patients with endometrial cancer were evaluated and analyzed in terms of glycolytic pathways, retrospectively. The genomic profile of the patients was the only data used and analyzed to explore whether there is a common pattern of gain or loss of the any glycolytic gene(s) locus in endometrial cancer.

Microarray-based comparative genomic hybridization (aCGH) has been routinely used for assessing the gain and loss of genomic regions in Kocaeli University Medical Genetics department . For this purpose, genomic DNA was isolated from patients

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Table 1. — *Glycolytic genes and their location on the genome. The highest number of the glycolytic genes are located on chromosomes 1 and 11 whereas chromosome 8, 14, and Y have none.*

Number of glycolytic genes on given chromosomes			
Chrosome	Number of Glycolytic Gene	Chrosome	Number of Glycolytic Gene
Chromosome 1	11	Chromosome 13	1
Chromosome 2	6	Chromosome 14	0
Chromosome 3	4	Chromosome 15	5
Chromosome 4	4	Chromosome 16	3
Chromosome 5	2	Chromosome 17	6
Chromosome 6	2	Chromosome 18	1
Chromosome 7	6	Chromosome 19	3
Chromosome 8	0	Chromosome 20	2
Chromosome 9	4	Chromosome 21	2
Chromosome 10	4	Chromosome 22	1
Chromosome 11	11	X	6
Chromosome 12	7	Y	0

Table 2. — *Displays the number of patients diagnosed with endometrial cancer with and without aberrations. Among a total of 33 patients, 33 had no copy number change, 21 had a copy number variation, and a total of 19 had multiple glycolytic gene copy number change.*

	Number of Patients
Without aberrations	33
With aberrations	21
With glycolytic gene aberrations	19
Total	54

using the DNeasy blood and tissue kit. After agarose gel and nanodrop measuring of the isolated DNA, qualified ones have been used along with the reference human DNA. Both reference and sample genomic materials were labelled following the CytoChip protocol and hybridized with aCGH microchips (Cytochip Focus Constitutional microarrays). The microchips were read by a microarray scanner and analyzed using BlueFuse Multi v2.2 software program CytoChip algorithm with fixed threshold. NCBI36 assembly data was used by the software. The array quality was checked by the software based on SD autosome/robust, percentage included clones, Mean Spot, Amplitude Ch1/Ch2, SBR Ch1/Ch2, and DLR Raw/Fused.

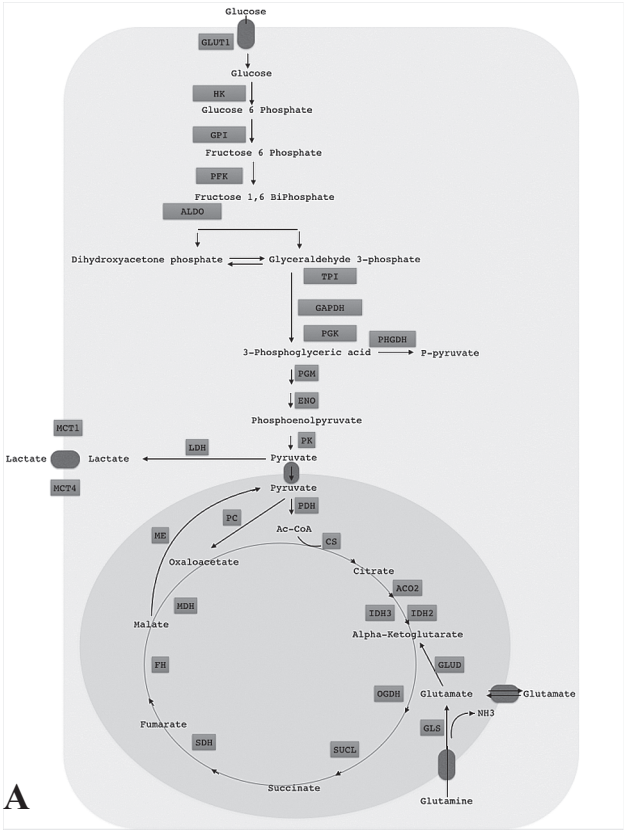
Glycolytic genes and their positions on the chromosomes were identified and listed from the NCBI website and the KEGG (Kyoto Encyclopedia of Genes and Genomes) database. Patient genomic gain and loss was analyzed using the glycolytic pathway genes' locations taken from the NCBI database using NCBI36 assembly data. Statistic measurements were applied using the copy numbers from the array and standard deviations were calculated.

Results

The number of genes involved in glycolysis based their locations in the genome were identified and listed in Table 1 according to the KEGG and NCBI databases. According to this list, chromosome 8 and chromosome Y do not contain any glycolytic genes, whereas chromosome 1 and chromosome 11 contain the highest number of glycolytic genes. The introduction of the oxygen into the atmosphere was about two billion years ago, and this suggests the first appearance of eukaryotes with aerobic metabolism [5]; however, it is not known how the chromosome evolution lineage has affected the distributions of positions of the glycolytic genes within the genome.

The general summary of chromosomal aberrations in the patients with endometrial cancer is shown in Table 2 based on their genomic laboratory reports. The total number of patients without chromosome aberrations was 33 out of 54 (61.3%) whereas that of with aberrations was 21 out of 54 (35.2%). Among the patients who had chromosomal aberrations, 19 of out of 21 (90.5%) had at least one glycolytic pathway gene(s) aberrations.

Within the population of the patients diagnosed with endometrial cancer, the copy number of succinate dehydro-



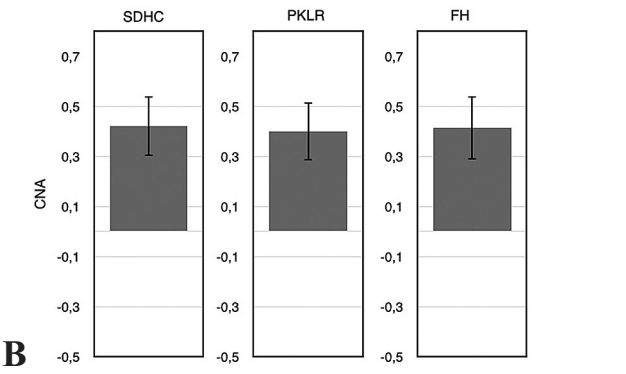


Figure 1. — A) Glycolytic pathway scheme. B) SDHC, PKLR, and FH copy number arrangement levels in endometrial cancer patients are shown.

genase C (SDHC), pyruvate kinase LR (PKLR), and fumarate (FH) were the most affected genes in the glycolytic pathway and all displayed duplications rather than deletion. SDHC and PKLR were observed as duplicated in 14 out of 21 (66.6%) patients with aberrations with glycolytic pathway genes, while FH duplication was seen in 12 (57.1%) cases. As depicted in Table 3, hexokinase (HK) forms of HK1 and HK2; phosphofructokinase (PFK) form of PFKP,

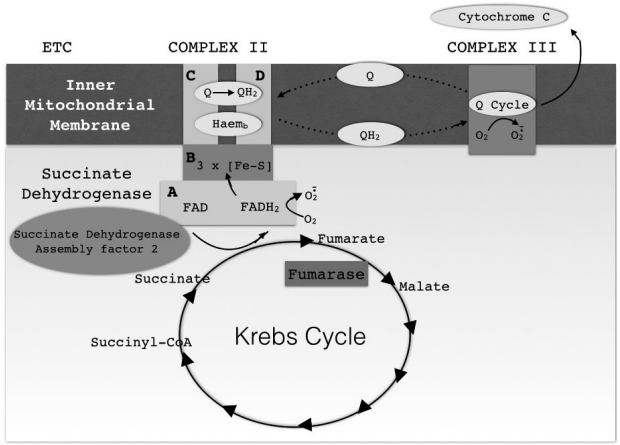


Figure 2. — Succinate dehydrogenase and its subunits are shown in an electron transport chain.

and glutamate dehydrogenase GLUD1 were also seen as duplicated in endometrial cancer patients.

In order to display the functional positions of SDHC, PKLR, and FH, the glycolytic pathway is shown in Figure 1. The lowest SDHC copy number was 0.28 and the highest was 0.65. The mean value for the copy number for SDHC was 0.421. Calculated standard deviation was 0.119. PKLR had lowest (0.28) and highest (0.65) copy numbers. The mean copy number 0.4 and standard deviation of PKLR copy number was 0.117. Lastly, FH lowest and highest copy numbers were 0.27 and 0.65, respectively. The mean copy number for FH was 0.415 and the standard deviation was 0.126. Taken together, the most recurrent copy number changes in glycolytic pathway genes in endometrial cancer patients were SDHC, PKLR, and FH. The significance of the increased copy number of these three genes in endometrial carcinoma remains to be explored.

Discussion

The succinate dehydrogenase enzyme (also known as succinate-ubiquinone oxydoreductase) is a highly conserved heterotetrameric protein complex, with SDHA and SDHB as catalytic subunits, which extend into the mitochondrial matrix and are anchored to the inner membrane by SDHC and SDHD [6, 7]. These latter subunits provide also the binding site for the ubiquinone (coenzyme Q10 or Q as shown in Figure 2) then reducing it to ubiquinol (QH_2). The SDH complex comprises mitochondrial complex II, which is involved in the Krebs cycle and in the electron transport chain (ETC) [8]. Complex II couples the oxidation of succinate to fumarate in the Krebs cycle with the electron transfer to the terminal acceptor ubiquinone in the ETC. Partial ubiquinone binding and stabilizing site of it is in SDHC along with SDHB and SDHD. As part of the mitochondrial electron transport chain, coenzyme Q10 ac-

Table 3. — *Chromosomal locations of glycolytic genes.*

Gene symbol	Gene full name	Gene ID	Location
GLUT1 (SLC2A1)	Solute carrier family 2 member 1	6513	1p34.2
HK1	Hexokinase 1	3098	10q22.1
HK2	Hexokinase 2	3099	2p12
HK3	Hexokinase 3	3101	5q35.2
HKDC1	Hexokinase domain containing 1	80201	10q22.1
GCK	Glucokinase	2645	7p13
GPI	Glucose-6-phosphate isomerase	2821	19q13.11
PFKL	Phosphofructokinase, liver type	5211	21q22.3
PFKM	Phosphofructokinase, muscle	5213	12q13.11
PFKP	Phosphofructokinase, platelet	5214	10p15.2
ALDOA	Aldolase, fructose-bisphosphate A	226	16p11.2
ALDOB	Aldolase, fructose-bisphosphate B	229	9q31.1
ALDOC	Aldolase, fructose-bisphosphate C	230	17q11.2
TPI1	Triosephosphate isomerase 1	7167	12p13.31
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	2597	12p13.31
GAPDHS	Glyceraldehyde-3-phosphate dehydrogenase, spermatogenic	26330	19q13.12
PGK1	Phosphoglycerate kinase 1	5230	Xq21.1
PGK2	Phosphoglycerate kinase 2	5232	6p12.3
PGM1	Phosphoglucomutase 1	5236	1p31.3
PGM2	Phosphoglucomutase 2	55276	4p14
PGM3	Phosphoglucomutase 3	5238	6q14.1
PGM5	Phosphoglucomutase 5	5239	9q21.11
ENO1	Enolase 1	2023	1p36.23
ENO2	Enolase 2	2026	12p13.31
ENO3	Enolase 3	2027	17p13.2
PKM	Pyruvate kinase, muscle	5315	15q23
PKLR	Pyruvate kinase, liver and RBC	5313	1q22
LDHA	Lactate dehydrogenase A	3939	11p15.1
LDHB	Lactate dehydrogenase B	3945	12p12.1
LDHC	Lactate dehydrogenase C	3948	11p15.1
UEVLD	UEV and lactate/malate dehyrogenase domains	55293	11p15.1
LDHAL6A	Lactate dehydrogenase A like 6A	160287	11p15.1
LDHAL6B	Lactate dehydrogenase A like 6B	92483	15q22.2
SLC16A1	Solute carrier family 16 member 1	6566	1p13.2
SLC16A4	Solute carrier family 16 member 4	9122	1p13.3
G6PD	Glucose-6-phosphate dehydrogenase	2539	Xq28
PGD	Phosphogluconate dehydrogenase	5226	1p36.22
TKTL1	Transketolase like 1	8277	Xq28
TKT	Transketolase	7086	3p21.1
TKTL2	Transketolase like 2	84076	4q32.2
TALDO1	Transaldolase 1	6888	11p15.5
PHGDH	Phosphoglycerate dehydrogenase	6227	1p12
PSAT1	Phosphoserine aminotransferase 1	29968	9q21.2
PSPH	Phosphoserine phosphatase	5723	7p11.2
PC	Pyruvate carboxylase	5091	11q13.2
PDHA1	Pyruvate dehydrogenase E1 alpha 1 subunit	5160	Xp22.12
PDHA2	Pyruvate dehydrogenase E1 alpha 2 subunit	5161	4q22.3
PDHB	Pyruvate dehydrogenase E1 beta subunit	5162	3p14.3
PDHX	Pyruvate dehydrogenase complex component X	8050	11p13
DLAT	Dihydrolipoamide S-acetyltransferase	1737	11q23.1
DLD	Dihydrolipoamide dehydrogenase	1738	7q31.1
CS	Citrate synthase	1431	12q13.3
ACO1	Aconitase 1	48	9p21.1
ACO2	Aconitase 2	50	22q13.2
ACO3 (IREB2)	Iron responsive element binding protein 2	3658	15q25.1
IDH1	Isocitrate dehydrogenase (NADP(+)) 1, cytosolic	3417	2q34
IDH2	Isocitrate dehydrogenase (NADP(+)) 2, mitochondrial	3418	15q26.1
IDH3A	Isocitrate dehydrogenase 3 (NAD(+)) alpha	3419	15q25.1
IDH3B	Isocitrate dehydrogenase 3 (NAD(+)) beta	3420	20p13

IDH3G	Isocitrate dehydrogenase 3 (NAD(+)) gamma	3421	Xq28
OGDH	Oxoglutarate dehydrogenase	4967	7p13
DLD	Dihydrolipoamide dehydrogenase	1738	7q31.1
PDHX	Pyruvate dehydrogenase complex component X	8050	11p13
SUCLG2	Succinate-CoA ligase GDP-forming beta subunit	8801	3p14.1
SUCLG1	Succinate-CoA ligase alpha subunit	8802	2p11.2
SUCLA2	Succinate-CoA ligase ADP-forming beta subunit	8803	13q14.2
SDHA	Succinate dehydrogenase complex flavoprotein subunit A	6389	5p15.33
SDHB	Succinate dehydrogenase complex iron sulfur subunit B	6390	1p36.13
SDHC	Succinate dehydrogenase complex subunit C	6391	1q23.3
SDHD	Succinate dehydrogenase complex subunit D	6392	11q23.1
FH	Fumarate hydratase	2271	1q43
MDH2	Malate dehydrogenase 2	4191	7q11.23
GLS	Glutaminase	2744	2q32.2
GLS2	Glutaminase 2	27165	12q13.3
GLUD1	Glutamate dehydrogenase 1	2746	10q23.2
GLUD2	Glutamate dehydrogenase 2	2747	Xq24
ME2	Malic enzyme 2	4200	18q21.2
ME3	Malic enzyme 3	10873	11q14.2
ACLY	ATP citrate lyase	47	17q21.2
ACACA	Acetyl-CoA carboxylase alpha	31	17q12
FASN	Fatty acid synthase	2194	17q25.3
SLC1A5	Solute carrier family 1 member 5	6510	19q13.32
SLC7A5	Solute carrier family 7 member 5	8140	16q24.2
GSS	Glutathione synthetase	2937	20q11.22
PPAT	Phosphoribosyl pyrophosphate amidotransferase	5471	4q12
GART	Phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase	2618	21q22.11
PFAS	Phosphoribosylformylglycinamidine synthase	5198	17p13.1
ATIC	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase	471	2q35
CAD	Carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase	790	2p23.3
DHODH	Dihydroorotate dehydrogenase	1723	16q22.2
UMPS	Uridine monophosphate synthetase	7372	3q21.2

cepts electrons from reducing equivalents generated during fatty acid and glucose metabolism and then transfers them to electron acceptors. At the same time, coenzyme Q10 transfers protons outside the inner mitochondrial membrane, creating a proton gradient across that membrane. The energy released when the protons flow back into the mitochondrial interior is used to form ATP [9, 10]. Mutations affecting the activity of SDH subunits in B, C, and D result in increased ROS production and enhanced tumorigenesis. Elevated rates of reactive oxygen species (ROS) have been detected in almost all cancers, where they promote many aspects of tumor development and progression [11]. Furthermore, loss of SDH leads to succinate build up. Hypoxia and succinate accumulation synergistically lead to hypermethylation of histones and DNA indirectly [12].

Overexpression of wild-type SDHC had no influence on the lifespan in *C. elegans* and overexpressed SDHC increased the amount of protein carbonyl compared to control, suggesting that deregulation of SDHC results in oxidative stress [13]. On the other hand in tumor cells, SDHC is thought to a be tumor suppressor; however, the tumor suppressor molecular mechanism of SDHC is yet to be defined. Hu *et al.* [14] analyzed more than 2,500 microarray using 22 different cancer-normal pairs and the meta analysis revealed that SDHC mRNA level has the highest expression fold change in female specific cancers compared to the other cancer types. Cervix squamous cell carcinoma samples displayed a 2.90 log2 fold higher differential expression for SDHC (p value= 3.4E-04). Ovary serous carcinoma showed a 1.72 (p value = 1.2E-02) (log2 scale) fold change for expression for SDHC.

Pyruvate kinase catalyzes the transfer of a phosphate group from phosphoenolpyruvate to ADP and converts to pyruvate and ATP in glycolysis. PK has different mammalian isoforms: PKM1, PKM2, and PKLR. Most adult tissues express PKM2; however, the other isoforms display tissue specificity. PKM1 is mostly expressed in tissues which have higher catabolism rates such as muscle, heart, and brain while PKLR is exclusively expressed in liver red blood cells [15]. PK activity is strictly regulated and an irreversible step in glycolysis after hexokinase and phos-

phofructokinase. The PK step is regulated by allosteric factors, covalent modifiers (phosphorylation), and hormones. Allosteric regulators of PK are alanine (a biosynthetic product of pyruvate) and ATP (negatively); and fructose-1,6-biphosphate (positively). Regulation through covalent modification of PK is via phosphorylation of the enzyme. High glucagon (low blood sugar) levels lead to PK phosphorylation causing restricted enzyme activity [16].

Inherited pyruvate kinase deficiency causes hemolytic anemia and leads red blood cells to break down easily. In this inherited disorder, pyruvate and lactate levels are lower than normal, while intermediates such as 1,3-bisphosphoglycerate (1,3 BPG) and phosphoenolpyruvate (PEP) accumulates. On the other hand, high levels of PKLR protein using transgenic expression in mice does not affect metabolic variables. Mice expressing high levels of PK have normal PK activity and ATP levels indicating that the transgenic expression of PK in these cells did not affect the biochemical balance of the energy pathway, probably due to delicate regulation of glycolytic pathway by other key enzymes, intermediary metabolites, and redox coenzymes. Serum levels of PK are measured as an indication of stability of the internal environment. Normal levels of serum PK were observed, indicating normal homeostatic balance and no side effects in leukocytes are produced by the increased PK expression through transgenic expression in mice [17].

Human protein atlases for normal and cancer tissues based on antibody staining and proteomics studies (proteinatlas.org) indicates that pathological protein expression of PKLR is linked with different cancer types including endometrial cancer [18–20]. Although very low, the liver and red blood cell specific forms of PKLR were found to be expressed in endometrial cancer tissue. Since PKLR is specific to liver and red blood cells, it remains to be explored why endometrial cancer tissue prefers to express a silenced gene.

Fumarase (fumarate hydratase) is an enzyme that catalyzes the reversible hydration/dehydration of fumarate to malate in citric acid cycle. Fumarase was identified as a mitochondrial tumour suppressor gene in families with the hereditary uterine leiomyomatosis and renal cell carcinoma (HLRCC) syndrome [21]. HLRCC syndrome is a genetic disorder and germline loss of function mutations of fumarase gene lead to an increased risk of cutaneous and uterine leiomyomas and renal cancer. Somatic loss of function of FH mutations are not common but are mostly seen in uterus leiomyomas [22].

FH which ubiquitously expressed throughout the body is also known as a tumor suppressor [23]. The increased genomic copy number of FH in the present study may be correlated with the mRNA level and might be consistent with the result of a Hu *et al.*'s retrospective study [24]. Their meta analysis showed that FH also has the highest expression fold change - like SDHC - in female specific cancers compared to the other cancer types. Cervix squamous cell carcinoma indicated 0.52 log2 fold higher differential expression for FH (p = 1.6E-01, respectively). Ovary serous carcinoma 1.08 log2 fold (p value = 4.5E-02) change for expression for FH. FH's role in female specific cancers is yet to be explored.

Conclusion

Multiple genes encoding glycolytic pathway enzymes are related to tumor metabolism including SDHB, SDHC, and succinate dehydrogenase subunits B,C,D (SDHD), FH, PK [6, 15, 22, 23, 25]. Copy number alteration of SDHC, FH, and PKLR glycolytic pathway genes seem to be recurrent in endometrial cancer patients. Many recurrent CNA cannot be fully explained by the presence of known cancer genes although oncogenes and tumor suppressors force and lead to some recurrent CNA in the tumor cells [2]. Analysis of different tumors can describe a defined CNA signature. This might be a predictive tool in the future for cancer patients [2]. However, the preference of cancer cells for predictive metabolic phenotypes and CNA still remains to be explored.

Since the discovery of Warburg effect in 1920s, the significance of metabolic reprogramming in carcinogenesis continues to grow and directs pharmacological drug design. Further research on tumor metabolism will shed light on how to most effectively and selectively destroy cancer cells.

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