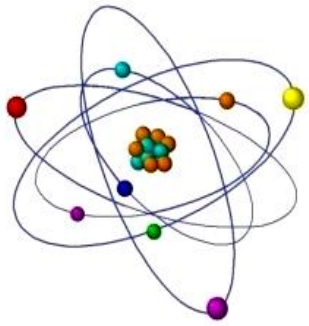
STUDYING THE IMPACT OF UBIQUITIN ON RADIATION-INDUCED DAMAGE TO GENES THROUGH BIOINFORMATIC METHODOLOGIES



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ABSTRACT: *Understanding the intricacies of cellular responses to radiation exposure is crucial for advancing radiobiology and developing effective strategies against radiation-induced pathologies. The annual radiation dose, sourced from natural and human-made sources, underscores the critical nature of this problem. Ubiquitination, as a pivotal process in protein regulation, holds the potential for alterations post-irradiation, impacting cellular recovery. The unique features of ubiquitin, beyond protein degradation, position it as one of the key players in comprehending cellular responses to radiation exposure.*
Our studies, employing bioinformatic methods, have revealed the potential use of ubiquitin in the context of radiation-induced cell exposure. We conducted an analysis to assess the commonality in the genetic spectrum between genes damaged by radiation and genes associated with ubiquitin. This study utilizes RNA sequencing to identify differentially expressed genes in mice subjected to irradiation. Our investigation delves into the impact of irradiation on ubiquitin-related genes, on various functions such as chromosome segregation and organelle fission. The heightened expression of genes associated with the cell cycle suggests the foundational role of ubiquitin-related genes in organism recovery after radiation exposure. The study not only contributes to advancements in radiobiology but also offers direction for developing preventive strategies against radiation-induced pathologies.

Key words: radiation exposure, bioinformatics, cellular responses, protein regulation

INTRODUCTION

Approximately 80% of the annual cumulative dose of background radiation received by an individual emanates from naturally occurring terrestrial and cosmic radiation sources. Additionally, on a daily basis, individuals may inhale or ingest radionuclides originating from natural sources present in soil, water, and air. Human-made sources of radiation exposure cover a spectrum ranging from nuclear power generation to various medical devices, including x-ray machines and Computed Tomography (CT) scanners [1]. Radiotherapy is a prevalent treatment for over half of cancer patients, using high doses of radiation to eradicate malignant cells. However, the associated side effects are a considerable concern. Consequently, any enhancements in the field of radiotherapy hold the potential to significantly benefit many people [2]. Hence, it is crucial to investigate not only the mechanisms underlying the impact of radiation but also the potential for regulating and reducing the negative effects of radiation on the body. Exploring causative associations within the responses of living organisms to radiation

affords an enhanced comprehension of risks associated with diverse manifestations of radiation exposure.

Ubiquitination is a pivotal facet in the regulation of protein metabolism. Ubiquitylation represents a post-translational modification where ubiquitin attaches to a target protein. Ubiquitin, a 76-amino acid protein, can exist either freely or be conjugated to a protein, either as a single ubiquitin (monoubiquitination) or as multiple ubiquitins (polyubiquitination). The ubiquitination pathway involves three enzymes: Ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin-protein ligase (E3). Ubiquitin modifications intricately regulate essential cellular processes, including proteasomal degradation, activation of cell signaling cascades such as NF-κB, protein trafficking, DNA repair, maintenance of genome integrity, control of the cell cycle, and programmed cell death. A deep understanding of ubiquitination is critically important in the context of the pathobiology of various human diseases [4, 5, 6].

The versatile characteristics of ubiquitination suggest that processes involving ubiquitin may undergo alterations following irradiation, thereby influencing the recovery process subsequent to radiation exposure.

In our previous work, we identified the effect of ubiquitin administration on the proliferation of blood cells after exposure to radiation in mice, compared with intact groups [7, 8, 9, 10].

To enhance the efficiency of our investigation, we employed computational technology, using a bioinformatic approach. Bioinformatics represents the convergence of biology and informatics, offering a potent approach equipped with techniques and specialized software tools for the analysis of biological data, such as the study of the human genome [11].

Differentially Expressed Genes analysis is an effective method to identify genes implicated in disease development, allowing us to find biological distinctions between healthy and pathological states [12]. This strategic utilization of computer technology not only expedites data analysis but also affords the advantage of handling vast datasets of genomic studies, thereby augmenting the overall efficiency of our research.

Our investigation includes a comparative analysis of the spectrum of genes altered by radiation with the genetic set involved in the ubiquitylation process. The importance of such comparative study lies in the identification of damaged components, providing insights that could be used in developing strategies in radiotherapy.

MATERIALS AND METHODS

To interpret the results, we employed RNA sequencing (RNA-seq) analysis data to identify differentially expressed genes in both irradiated and non-irradiated conditions among young mice (2-3 months old). Although our working group had previously analyzed this data, we found it useful in elucidating the influence of ubiquitin on the organism post-irradiation [13, 14].

The information pertaining to ubiquitin-related genes was retrieved from an open-source database [15]. Molecular pathways identification was conducted using the David Genes bioinformatics platform and the KEGG PATHWAY database. For data processing and figure generation, statistical packages in R and Python were utilized.

RESULTS

In our prior investigation, we analyzed 322 differentially expressed genes, considering a threshold of expression levels exceeding ±1. Out of these, 212 genes associated with the ubiquitin and ubiquitination processes were identified. Based on the expression level higher than ±1.5 we identified 74 genes from this data set. The information regarding ubiquitin-related genes was sourced from the Gene Cards database [15].

According to our findings, the majority of ubiquitin-related genes are upregulated, with only three cases of downregulated genes. Notably, pronounced alterations in ubiquitin-related genes of irradiated mice were observed in chromosomes 1, 2, 7, and 11. To elucidate the gene expression patterns in this context, we conducted Gene Ontology analysis (GO) utilizing R bioinformatic packages. GO is a widely used tool to specify gene involvement in terms of cellular location, molecular function, and biological processes. For GO analysis 10 additional genes with expression levels lower than ±1.5 were used.

The biological processes (BP), molecular functions (MF), and cellular components (CC) associated with the obtained gene set are represented in Figure 1, Figure 2, and Figure 3 respectively. We chose genes with p. value < 0.0001 and the number of genes participating in the processes no less than 8.

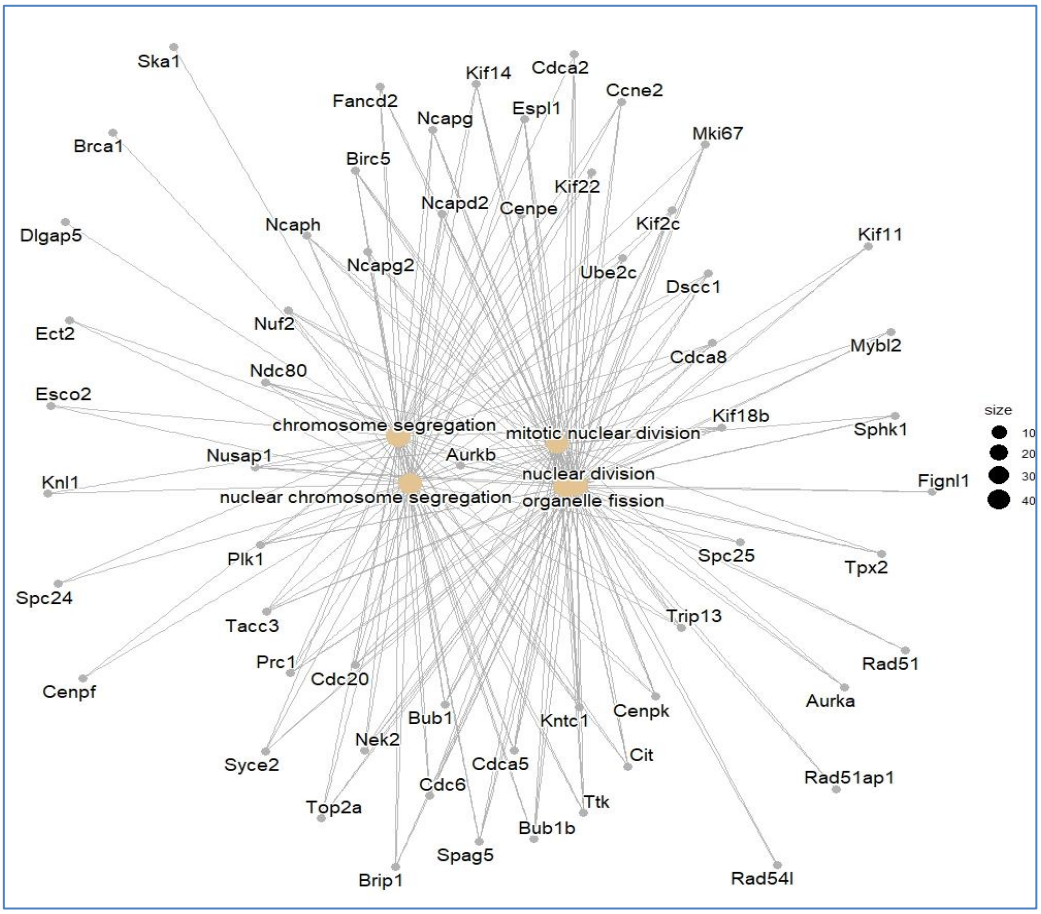

Figure 1. Biological processes (BP) associated with obtained genes set

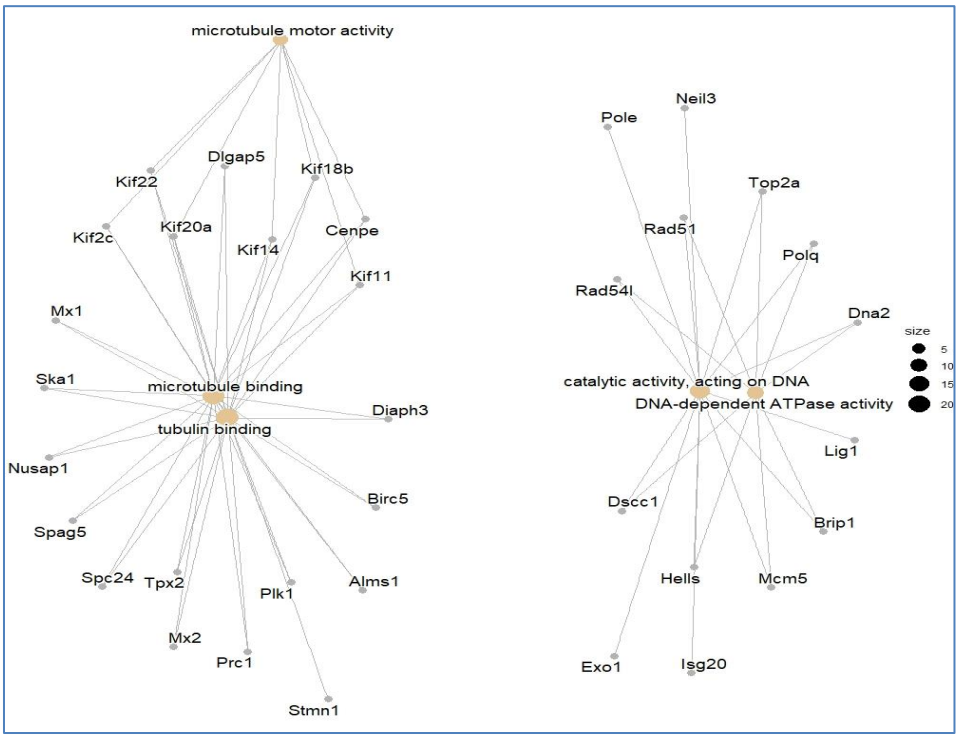

Figure 2. Molecular functions (MF) associated with obtained genes set.

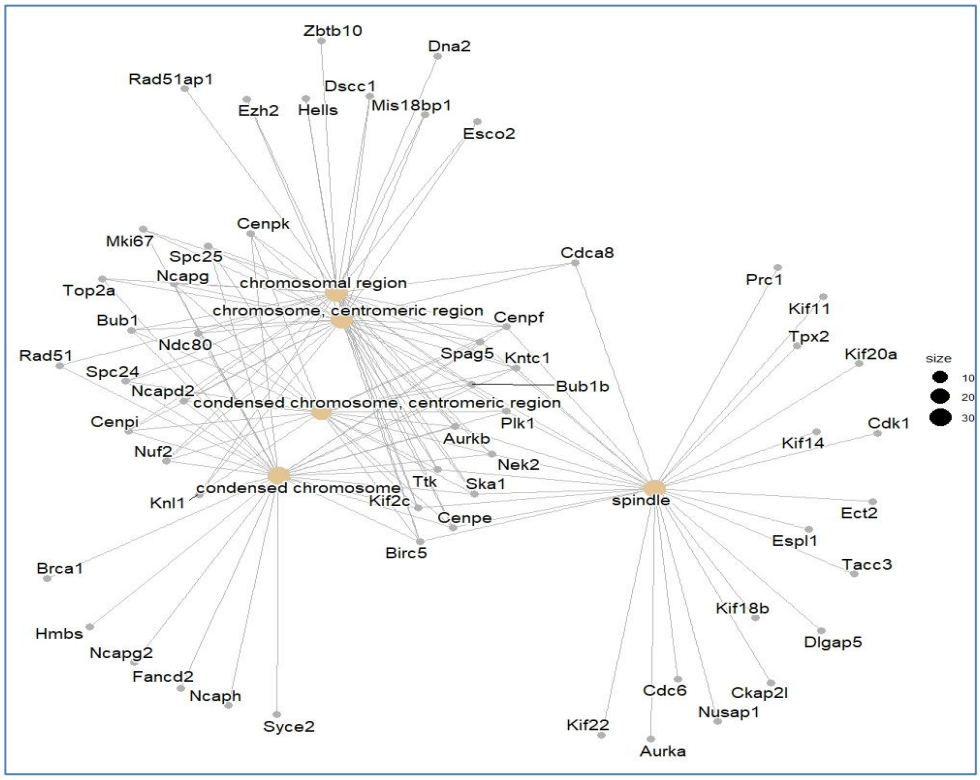

Figure 3. Cellular components (CC) associated with obtained genes set.

We quantified genes involved in biological processes (BP), molecular functions (MF), and cellular components (CC) in gene ontology analysis. Utilizing the entire gene set common with ubiquitin-related genes, regardless of their expression levels, we identified 76 genes for BP, 75 for MF, and 60 for CC. Notably, 28 genes actively participated in all three processes: 'Tpx2', 'Aurkb', 'Kif22', 'Top2a', 'Aurka', 'Kif18b', 'Ect2', 'Dlgap5', 'Ttk', 'Cdk1', 'Bub1', 'Spc24', 'Dscc1', 'Ncapg2', 'Nek2', 'Kntc1', 'Rad51', 'Nusap1', 'Plk1', 'Bub1b', 'Kif14', 'Birc5', 'Ska1', 'Kif11', 'Spag5', 'Cenpe', 'Prc1', and 'Kif2c'.

This data suggest that the pathological condition induced by irradiation leads to a disruption in the cell division process. This observation indicates alterations in protein functions, with some of their genes exhibiting atypical expression levels. A comprehensive investigation, including RNA sequencing under narrower conditions of the experiment, could provide further insights into resolving this matter.

DISCUSSION

This bioinformatics project aimed to discern alterations in gene expression following radiation exposure, to broaden insights into the role of ubiquitin in diverse biological processes.

The unique properties of ubiquitin have positioned it as the focal point of our investigation. Beyond its role in protein degradation, ubiquitin exhibits the capability to post-translationally modify proteins, and form ubiquitin conjugates. This multifunctionality potentially exerts a profound impact on normal cellular functions [16]. Moreover, these distinctive features of ubiquitin suggest its participation as one of the contributors to cellular processes following radiation exposure.

Bioinformatics investigations of genes and Gene Ontology (GO) analysis, focusing on ubiquitin-related genes altered by irradiation, reveal significant impacts on various functions and processes. The affected biological processes include chromosome segregation, nuclear chromosome segregation, mitotic nuclear division, nuclear division, and organelle fission. In terms of molecular functions, alterations are observed in microtubule binding, tubulin binding, catalytic activity acting on DNA, and DNA-dependent ATPase activity. The changes in the expression of these genes manifest in the chromosomal centromeric region, spindle, and condensed chromosome centromeric region.

These processes are fundamental to cell formation and functionality, and their disturbances observed in this study are attributed to irradiation. The obtained information aligns with well-known effects of ionizing radiation, such as apoptosis-induced cell death and irreversible arrest of the G1/S cycle (G1/S arrest). These effects are characterized by the loss of normal nuclear structure, coupled with DNA degradation [17, 18].

Let us delve into the intricate processes governed by genes exhibiting differential expression in irradiated mice. During the accurate segregation of chromosomes, kinetochores attach to microtubules coming from opposing poles of the spindle, forming bioriented chromosomes on the metaphase spindle. Positively expressed in our study, Survivin (Birc5) and Aurora B kinase form a chromosome passenger complex, regulating this critical process. The deubiquitylation enzyme hFAM plays a role in dissociating Survivin from centromeres, while the ubiquitin-binding protein Ufd1 is essential for the association of Survivin with centromeres.

Consequently, ubiquitin serves as a regulatory factor ensuring the precise targeting of Survivin and Aurora B (Aurkb) to centromeres [19]. Moreover, the Linear Ubiquitin Chain Assembly Complex (LUBAC) catalyzes linear ubiquitination, facilitating chromosome congression and dynamic alignment by linking the dynamic kinetochore microtubule receptor CENP-E to the static KMN network. The KMN network is a multiprotein assembly component crucial for establishing kinetochore-microtubule junctions [20]. These detailed mechanisms highlight the intricate regulatory role of ubiquitin in orchestrating fundamental processes like chromosome segregation and alignment in response to irradiation. Considering ubiquitin's potent regulatory role, changes in its functions due to irradiation may profoundly impact the above-mentioned processes.

Upon examination of obtained protein set and their alignment with KEGG pathways, specifically those related to the cell cycle (Figure 3.), it was found that 23 of the identified genes, derived from gene expression analysis, are involved in the cell cycle, including the MAPK signaling pathways. Significantly, all these genes exhibit an increased expression in our study. This heightened expression implies a potential response aimed to initiate the restoration of cells lost due to radiation-induced cell death.

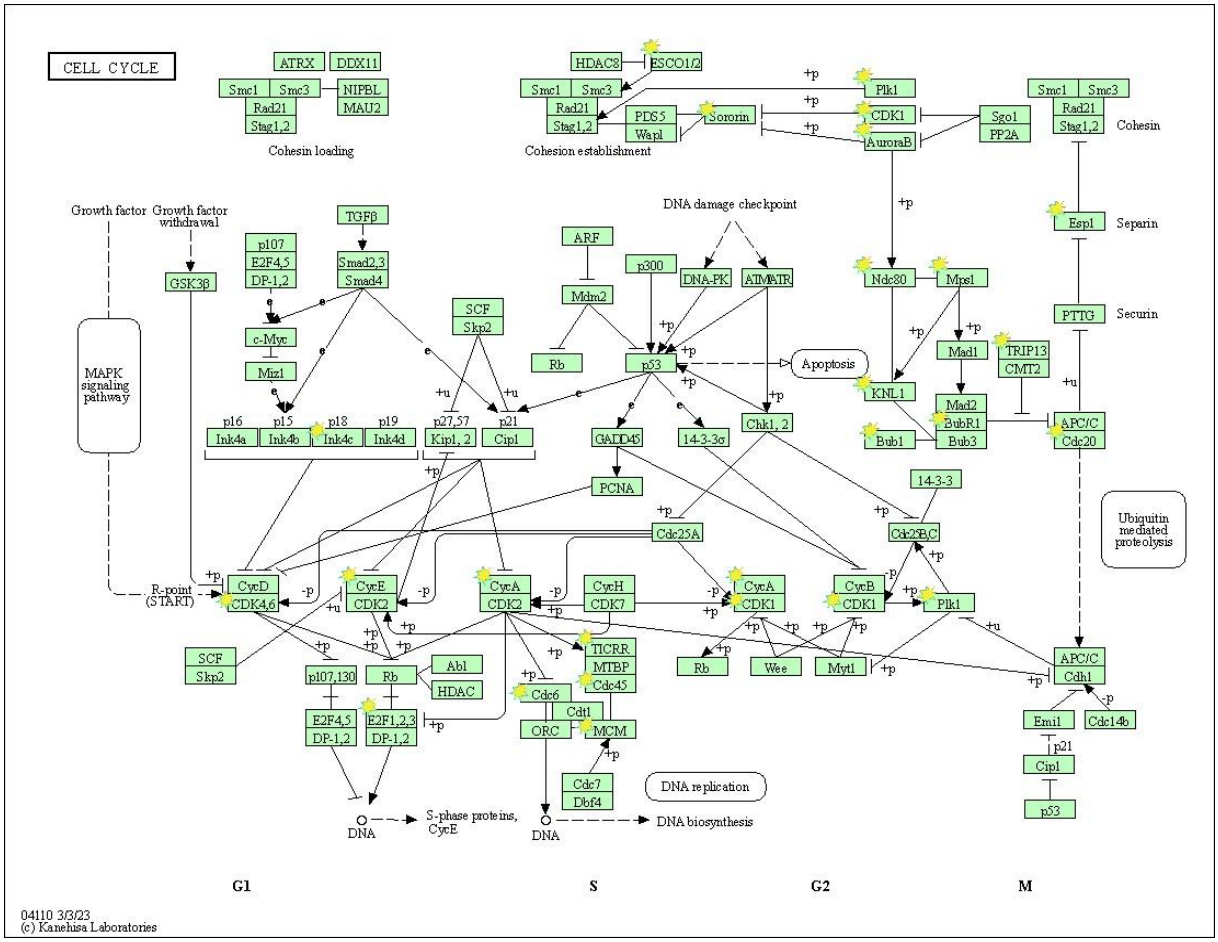

Figure 3. Cell cycle pathways. Asterisks indicate genes with the levels of expression changed by irradiation according to our study (the figure was generated based on our data using the David Genes bioinformatics platform and the KEGG PATHWAY database)

Activation of signaling pathways such as MAPK, PI3K, Wnt, and Sonic Hedgehog (SHH) is known to induce proliferation in various cell types [21]. Among these, the Ras/Raf/MAPK (MEK)/ERK pathway is a pivotal signaling cascade within the MAPK transduction pathways [22]. It is evident that the damage inflicted upon proteins involved in these signaling pathways by irradiation may be one of cases of the adverse effects of radiation on the organism.

Our investigation extends beyond the well-established effects of radiation, such as apoptosis and G1/S arrest, to propose a potential involvement of the MAPK signaling pathway. The comparative analysis and utilization of the KEGG pathways database highlight an association between irradiation and alterations in this crucial pathway governing cell proliferation and expression.

While the direct influence of ubiquitin remains unexplored in our study, it is crucial to emphasize the foundational role of ubiquitin-related genes in our computational analysis and the identification of the coincidences with known processes in which they participate. The possibility of changes in ubiquitylation and modifications of proteins under the influence of irradiation suggests a disruption in protein regulation. Our findings warrant further exploration through comprehensive studies employing advanced techniques like RNA sequencing and targeted protein quantification.

In essence, our study lays the groundwork for future research endeavors aimed at unraveling radiation-induced cellular responses and underscores the potential significance of the MAPK signaling pathway and ubiquitin-related processes in this context.

Future investigations under more refined conditions, including specific animal groupings and varied irradiation doses, hold promise for a deeper understanding of the intricate mechanisms of radiation exposure.

CONCLUSIONS

1. In accordance with our investigations, the commonality of the genetic spectrum between genes susceptible to radiation-induced damage and ubiquitin-related genes has been established. This observation pertains to 65% of the genes with altered expression subsequent to exposure to radiation. In the course of our research, 84 genes were selected, of which 74 exhibited a deviation in expression levels no less than ±1.5. Additionally, 10 genes were identified through the application of Gene Ontology (GO) analysis.
2. Gene Ontology (GO) analysis was applied to the obtained gene set, revealing insights that suggest alterations in gene expression may potentially stem from disruptions in the cellular division process and associated mechanisms. We interpret this observation as evidence of the potential of ubiquitin in mitigating the negative effects of radiation on the human body.
3. The gene set was studied using the KEGG database, allowing us to conclude that the radiation-induced disturbance in the cell cycle, considering ubiquitin-related genes, may correspond to an interference with the MAPK molecular pathway. Disruption of this molecular pathway is implicated in various pathologies.

Our investigation not only contributes to the understanding of radiobiology but also offers avenues for developing targeted interventions and preventive strategies against radiation-induced pathologies.

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