Clinical, Cosmetic and Investigational Dermatology

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ORIGINAL RESEARCH

Cathepsins and Skin Cancer (Malignant Melanoma, Basal Cell Carcinoma, and Squamous Cell Carcinoma): Insight From Genetic Correlation and Mendelian Randomization

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Background: Multiple studies have indicated that cathepsins (Cats) play a crucial role in the development and progression of skin cancer. However, most of these studies are observational and may be influenced by external variables, necessitating further research to establish causal relationships.

Methods: We conducted a two-sample, two-way Mendelian randomization (MR) study utilizing pooled data from genome-wide association studies (GWAS) to evaluate the causal association between 9 Cats (Cat-B, E, F, G, H, L2, O, S, and Z) and 3 types of skin cancer, including malignant melanoma (MM), basal cell carcinoma (BCC), and squamous cell carcinoma (SCC). Our analysis employed several methods, including inverse variance weighting (IVW), MR-Egger, weighted median, Cochran's Q test, the MR-Egger intercept test, and leave-one-out sensitivity analysis. Furthermore, bioinformatics analysis of loci linked to Cats and skin cancer was performed to explore potential molecular mechanisms.

Results: Genetically predicted increases in Cat-F and Cat-O levels were found to be correlated with a higher risk of BCC, while increased levels of Cat-L2 and Cat-O were associated with a reduced incidence of SCC. Bioinformatics analysis suggested that differentially expressed genes located near Cats-related loci could potentially influence BCC and SCC by modulating relevant signaling pathways and the tumor microenvironment.

Conclusion: Our research indicated a causal link between Cats and skin cancer. By conducting a bioinformatic analysis of genetic loci related to Cats and skin cancer, we were able to gain a better understanding of the potential molecular mechanisms driving this association. This research can provide valuable insights into the diagnosis and treatment of skin cancer.

Keywords: Cathepsins, Skin cancer, Genome-wide association study, Mendelian randomization, Bioinformatic analysis

Introduction

Skin cancer, including malignant melanoma (MM), basal cell carcinoma (BCC), and squamous cell carcinoma (SCC), are a leading cause of cancer-related deaths worldwide. The incidence of skin cancer is on the rise, posing a significant threat to human health.[1,2] Various risk factors contribute to the development and advancement of these tumors, with one critical factor being the ability of cancer cells to maintain internal equilibrium.[3] Proteins play a crucial role in regulating the metabolic stability of cancer cells, especially the proteolytic system, which promotes cancer cell proliferation, invasion, and metastasis. Among the essential protein-hydrolyzing enzymes in mammals are cathepsins (Cats), found both intracellularly and extracellularly. Cats are involved in a wide range of physiological and pathological processes, such as regulating autophagy, apoptosis, maintaining the balance of the extracellular matrix, antigen presentation, viral infections, inflammation, and the initiation and progression of various malignant tumors. The function of Cats in these

Received: 21 October 2024
Accepted: 27 February 2025
Published: 12 March 2025

processes highlights their significance in understanding the mechanisms underlying tumor development and progression in skin cancer.[4–6]

Prior researchers have found a clear link between Cats and the development of various malignant tumors, including esophageal cancer,[7] breast cancer,[8] and liver cancer.[9] A recent study using Mendelian randomization (MR) has indicated that high levels of Cat-G and Cat-B could increase the risk of hepatocellular carcinoma and biliary tract cancer, respectively.[10] Research has also indicated a link between Cats and skin cancer, especially Cat-B, Cat-L, and Cat-K, which affected invasion, metastasis, and poor prognosis in MM,[11–13] BCC,[14,15] and SCC.[16–18] In addition, Cat-D is associated with MM[19] and SCC.[16] Nonetheless, many of these investigations rely on observation and are subject to potential biases, underscoring the necessity for more robust study frameworks to establish a causal connection between Cats and skin cancer.

MR is a genetic epidemiology approach based on whole genome sequencing data that utilizes instrumental variables to uncover causal relationships and deal with confounding factors.[20] Its design principle: according to Mendel's law of inheritance, alleles from parents are randomly assigned to offspring during inheritance, and different genotypes of offspring determine different intermediate phenotypes.[21] The association of genes with disease outcomes is not confounded by confounding factors such as postnatal environment and is not plagued by reverse causation, thus genes can be used as a valid instrumental variable.[22] The purpose of our research was to explore the causality between Cats and skin cancer by analyzing 9 Cats and 3 skin cancer-associated genetic variants obtained from Genome-Wide Association Studies (GWAS). The investigation was performed by utilizing a two-sample bidirectional MR method. Our study provides novel perspectives into the causal relationship between Cats and skin cancer.

Materials and Methods

Study Design

To find the causal association between the 9 Cats (Cat-B, E, F, G, H, L2, O, S, and Z) and skin cancer (MM, BCC, and SCC), we utilized a two-sample MR method. Our study design is clearly described in Figure 1. Our research used SNPs as instrumental variables to assess the casual association between exposure (9 Cats) and outcome (MM, BCC, and SCC). Adherence to the basic assumptions of MR analysis is crucial for accurately assessing the causal relationship between genetic variation, exposure, and outcome in research studies.[21] The first assumption is correlation, which establishes a strong link between genetic variance and the exposure being studied. This correlation ensures that genetic influences on exposure factors are properly reflected in the analysis. The second assumption is independence, which indicates that the genetic variance is not affected by any confounding variables that could distort the association between the exposure and the outcome. Independence is essential for eliminating the possibility of skewed results and ensuring accurate conclusions. The third assumption is exclusion of restriction, which suggests that genetic variance affects the outcome only through the specific exposure factors under study. By adhering to these three assumptions, researchers can establish a clear causal relationship between genetic variation and outcomes, attributing any effects solely to the exposure factors being examined. In conclusion, the successful application of MR analysis hinges on meeting the criteria of correlation, independence, and exclusion restrictions. By upholding these fundamental assumptions, researchers can confidently evaluate the causal impact of genetic variations on exposure and outcomes in their studies. It is through the adherence to these principles that the validity and reliability of MR analyses can be ensured, leading to accurate and meaningful conclusions in the field of academic research.

Data Collection

Data on the 9 Cats level were sourced from the INTERVAL study,[23] which involved 3301 individuals of European descent. Summary statistics for skin cancers were extracted from various GWAS databases. MM genetic variation data (GWAS ID: ieu-b-4969) was publicly accessed at https://www.ebi.ac.uk/gwas, comprising of 375,767 samples (3751 cases and 372,012 controls). Genetic variation data for BCC (GWAS ID: GCST90013410) and SCC (GWAS ID: GCST90041917) were openly available at https://gwas.mrcieu.ac.uk, including 392,871 samples (17,416 cases and 375,455 controls) and 456,276 samples (557 cases and 455,719 controls) respectively. All data for this MR analysis

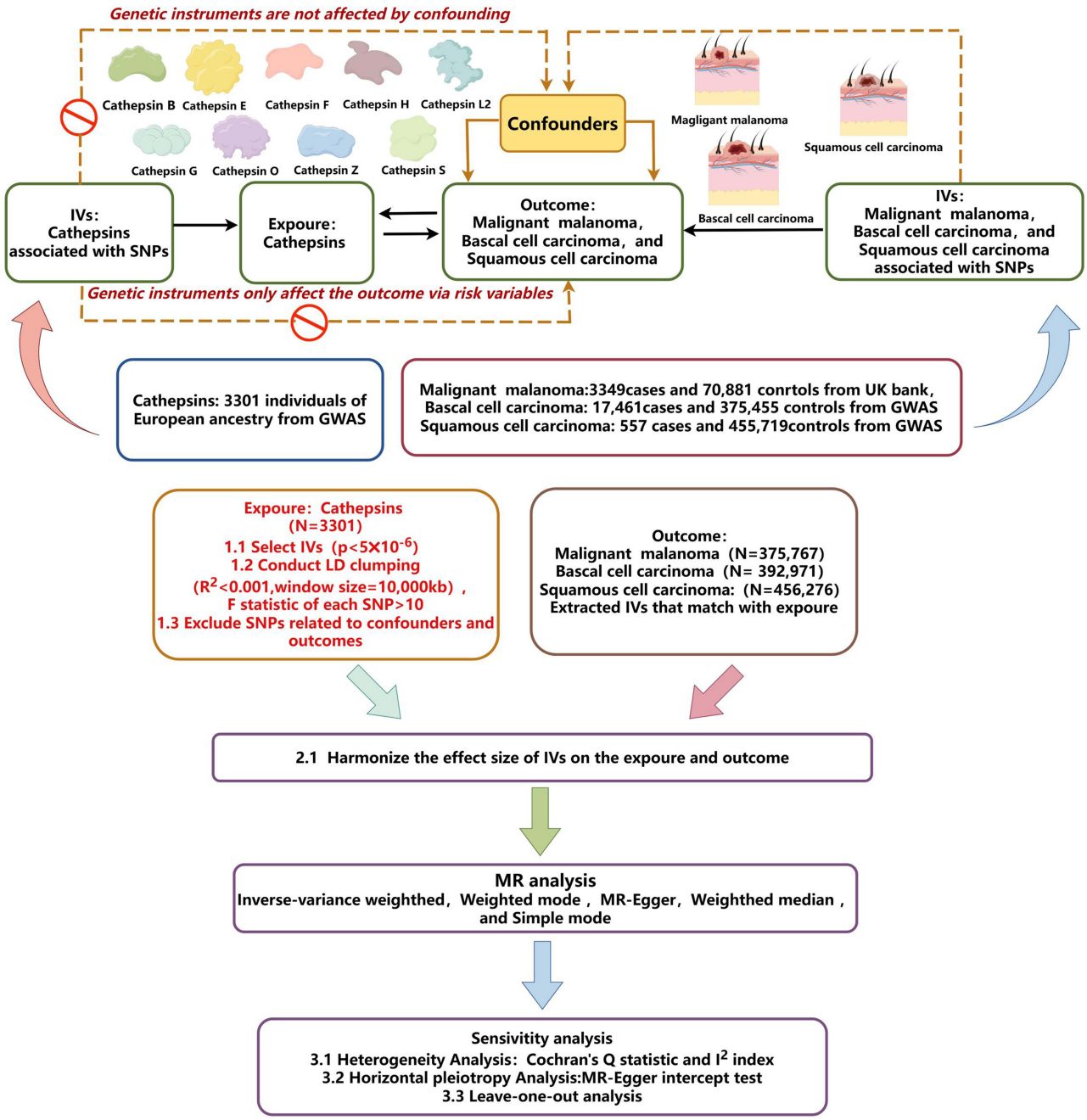

Figure 1 The design of bidirectional Mendelian randomization (MR) study by Figdraw.

were exclusively from European populations and had been scrutinized and approved by the relevant institutional ethical review boards. Thus, no additional ethical approvals or licenses were necessary for this study.

The Selection of Instrumental Variables

To explore the potential link between 9 Cats and 3 skin cancers, a thorough screening process was carried out to determine the instrumental variables (IVs) while also implementing measures to ensure data integrity. When conducting a forward analysis, our initial step involved identifying genome-wide SNPs from the GWAS database by applying a stringent threshold of $P < 5\times10^{-8}$. However, due to a limited number of SNPs meeting this criterion for both exposures and outcomes (less than 3), we adjusted our significance threshold to $P < 5\times10^{-6}$ for the selection of IVs related to the 9

Cats. Conversely, for the reverse analysis, a slightly less strict significance threshold of $P < 1\times10^{-5}$ was used in the selection process for IVs associated with the 3 cases of skin cancer. After this selection process, SNPs were grouped based on the European 1000 Genomes Project reference panel to evaluate their independence ($r^2 < 0.01$ and clump distance = 10,000 kb). IVs displaying weak F-statistics below 10 were excluded from the analysis due to their lack of robustness, underscoring the importance of utilizing strong IVs in scientific research. Overall, the meticulous and systematic approach taken in selecting IVs and conducting quality control measures to explore the correlation between felines and skin cancer aimed to ensure the reliability and validity of the results obtained. The emphasis on excluding weak instrumental variables highlights the significance of using sturdy IVs to establish a strong basis for data analysis and interpretation.

Statistical Analysis

In this MR investigation, we employed the software RStudio (version 4.3.2) to explore the potential link between Cats and susceptibility to skin cancer. The primary analytical approach utilized was IVW to assess the influence of exposure on the outcome. To guarantee the precision of causal estimates, adherence to the three core principles of MR investigations was imperative. Despite the effectiveness of IVW, it does possess certain limitations, particularly due to its reliance on assumptions and its capacity to consider confounding factors solely in the absence of horizontal pleiotropy. When employing the IVW method, it is critical to factor in the potential repercussions stemming from horizontal pleiotropy. Therefore, A number of MR methods were used in this study to test the robustness of the IVW method such as MR-Egger, weighted mode, simple mode, and weighted median.[24] The IVW method and MR-Egger regression were used to test for heterogeneity, while Cochran's *Q* test was used to quantify heterogeneity. The intercept term of the MR-Egger regression indicates the average multinomially of the IVs. In addition, A leave-one-out analysis was applied as a sensitivity analysis to further evaluate the causal results derived from the IVW method. This method was also used in this study to determine the robustness and consistency of the results.[25]

Bioinformatics Analysis

RStudio (version 4.3.2) was used for bioinformatics analysis to explore the molecular pathways involved in Cats' role in skin cancer development and progression. Databases available online were employed to pinpoint genes that are closely linked to both Cats and skin cancer, and these genes underwent enrichment analysis using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. The Genomic, Pharmacogenomic, and Immunogenomic Gene Set Cancer Analysis (GSCA) platform (https://guolab.wchscu.cn) was utilized to merge clinical data with knowledge on small molecule drugs, leading to the discovery of potential biomarkers and treatment options for future clinical trials. GSCA was also used to reveal the relationship between drug response and genes enriched in shared loci between Cats and skin cancer.

Results

Causal Relationship Between 9 Cats and 3 Skin Cancers

We systematically evaluated the casual effect between Cats (Cat-B, Cat-E, Cat-F, Cat-G, Cat-H, Cat-L2, Cat-O, Cat-S, and Cat-Z) and skin cancer (MM, BCC, and SCC) through a two-sample MR analysis. The primary research methodology utilized in our investigation was the IVW approach, a reliable method for meta-analyzing the effects of various genetic variants to produce causal estimates free from directional pleiotropy. The results of univariate MR analysis showed a causality between the Cats and 3 skin cancers. In our forward MR analysis, we identified a causal link between Cats (Cat-F and Cat-O) and the risk of BCC. In addition, we found a causal connection between Cats (Cat-L2 and Cat-O) and susceptibility to BCC, while no specific Cat was determined to be causally related to MM risk. Specifically, higher levels of Cat-F and Cat-O were related with increased BCC risk (odds ratio, OR: 1.0466, 95% confidence interval, CI: 1.0004–1.0948, p = 0.0478; OR: 1.0890, CI: 1.0168–1.1664, p = 0.0148) as shown in Figure 2. Conversely, elevated levels of Cat-O and Cat-L2 were linked to a lower risk of SCC (OR: 0.7100, CI: 0.5144–0.9827, p = 0.0388; OR: 0.7052, CI: 0.5161–0.9637, p = 0.0283) as illustrated in Figure 3. Furthermore, there was no observed association between

outcome	exposure	method	nsnp	b	OR(95%CI)	pval
basal cell carcinoma	Cathepsin F	MR Egger	9	0.05300113	1.05(0.93 to 1.20)	0.44555159
basal cell carcinoma	Cathepsin F	Weighted median	9	0.01503291	1.02(0.95 to 1.08)	0.64196625
basal cell carcinoma	Cathepsin F	Inverse variance weighted	9	0.04552620	1.05(1.00 to 1.09)	0.04780717
basal cell carcinoma	Cathepsin F	Simple mode	9	0.03958200	1.04(0.94 to 1.15)	0.47038972
basal cell carcinoma	Cathepsin F	Weighted mode	9	0.01466794	1.01(0.93 to 1.11)	0.75025154
basal cell carcinoma	Cathepsin O	MR Egger	7	0.06354187	1.07(0.88 to 1.29)	0.54698170
basal cell carcinoma	Cathepsin O	Weighted median	7	0.05049639	1.05(0.96 to 1.15)	0.26878022
basal cell carcinoma	Cathepsin O	Inverse variance weighted	7	0.08528817	1.09(1.02 to 1.17)	0.01482864
basal cell carcinoma	Cathepsin O	Simple mode	7	0.04539648	1.05(0.92 to 1.19)	0.50899977
basal cell carcinoma	Cathepsin O	Weighted mode	7	0.04539648	1.05(0.92 to 1.18)	0.50014197

P<0.05 was considered statistically significant

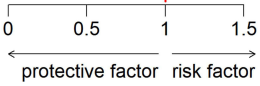

Figure 2 Bidirectional MR estimates of Cats and BCC (IVW, Weighted median, Weighted mode,MR-egger, Simple mode).

outcome	exposure	method	nsnp	b	OR(95%CI)	pval
squamous cell carcinoma	Cathepsin O	MR Egger	11	-0.2004749	0.82(0.37 to 1.82)	0.63484211
squamous cell carcinoma	Cathepsin O	Weighted median	11	-0.1736743	0.84(0.55 to 1.27)	0.41249709
squamous cell carcinoma	Cathepsin O	Inverse variance weighted	11	-0.3410949	0.71(0.51 to 0.98)	0.03883909
squamous cell carcinoma	Cathepsin O	Simple mode	11	-0.2115775	0.81(0.40 to 1.66)	0.57583305
squamous cell carcinoma	Cathepsin O	Weighted mode	11	-0.2047299	0.81(0.39 to 1.69)	0.59391692
squamous cell carcinoma	Cathepsin L2	MR Egger	10	-0.4948169	0.61(0.30 to 1.23)	0.20601083
squamous cell carcinoma	Cathepsin L2	Weighted median	10	-0.4269864	0.65(0.42 to 1.01)	0.05312733
squamous cell carcinoma	Cathepsin L2	Inverse variance weighted	10	-0.3492215	0.71(0.52 to 0.96)	0.02839746
squamous cell carcinoma	Cathepsin L2	Simple mode	10	-0.5217792	0.59(0.31 to 1.13)	0.14528993
squamous cell carcinoma	Cathepsin L2	Weighted mode	10	-0.5614444	0.57(0.31 to 1.05)	0.10456946

P<0.05 was considered statistically significant

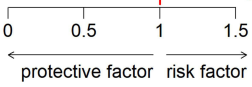

Figure 3 Bidirectional MR estimates of Cats and SCC (IVW, Weighted mode, MR-egger, Simple mode).

changes in Cat levels and MM risk as presented in Supplementary Table 1. Our IVW analysis did not find any causal association between other Cats and the occurrence of the 3 skin cancers (Supplementary Tables 1–3).

While IVW is a valuable tool, it has shortcomings as it relies heavily on the assumptions mentioned above and can only consider confounding factors in the absence of pleiotropy. Therefore, it is essential to think about the influence of potential pleiotropy on results when using this method. Then, we performed sensitivity analysis using MR-Egger to assess the credibility and consistency of our result. Utilizing the MR-Egger method, we detected horizontal pleiotropy (significance at $P < 0.05$). Moreover, the Cochran's Q test was carried out to identify heterogeneity within the chosen SNPs (significance at $P < 0.05$). The MR-Egger intercept was also employed for detecting horizontal pleiotropy. The analysis of both Cochran's Q and MR-Egger's intercepts revealed no evidence of heterogeneity or pleiotropy in the causal associations (Supplementary Tables 4 and 5). The scatterplot is shown in Figure 4 and the finding of the leave-one-out analysis presents the robustness of the results, as shown in Figure 5.

Causal Relationship Between 3 Skin Cancers and 9 Cats

Through reverse Mendelian randomization analysis, we investigated the relationship between skin cancer as exposure factors and of Cats as outcomes. Our findings did not reveal any evidence of reverse causality between skin cancers and Cats (Supplementary Table 6).

Differential Gene Expression Analysis of Near Genetic Loci

A comprehensive analysis of genetic loci related to Cats and skin cancer was conducted to explore the potential correlation between the two variables. The investigation found that 39 genes were linked with promoting Cats in

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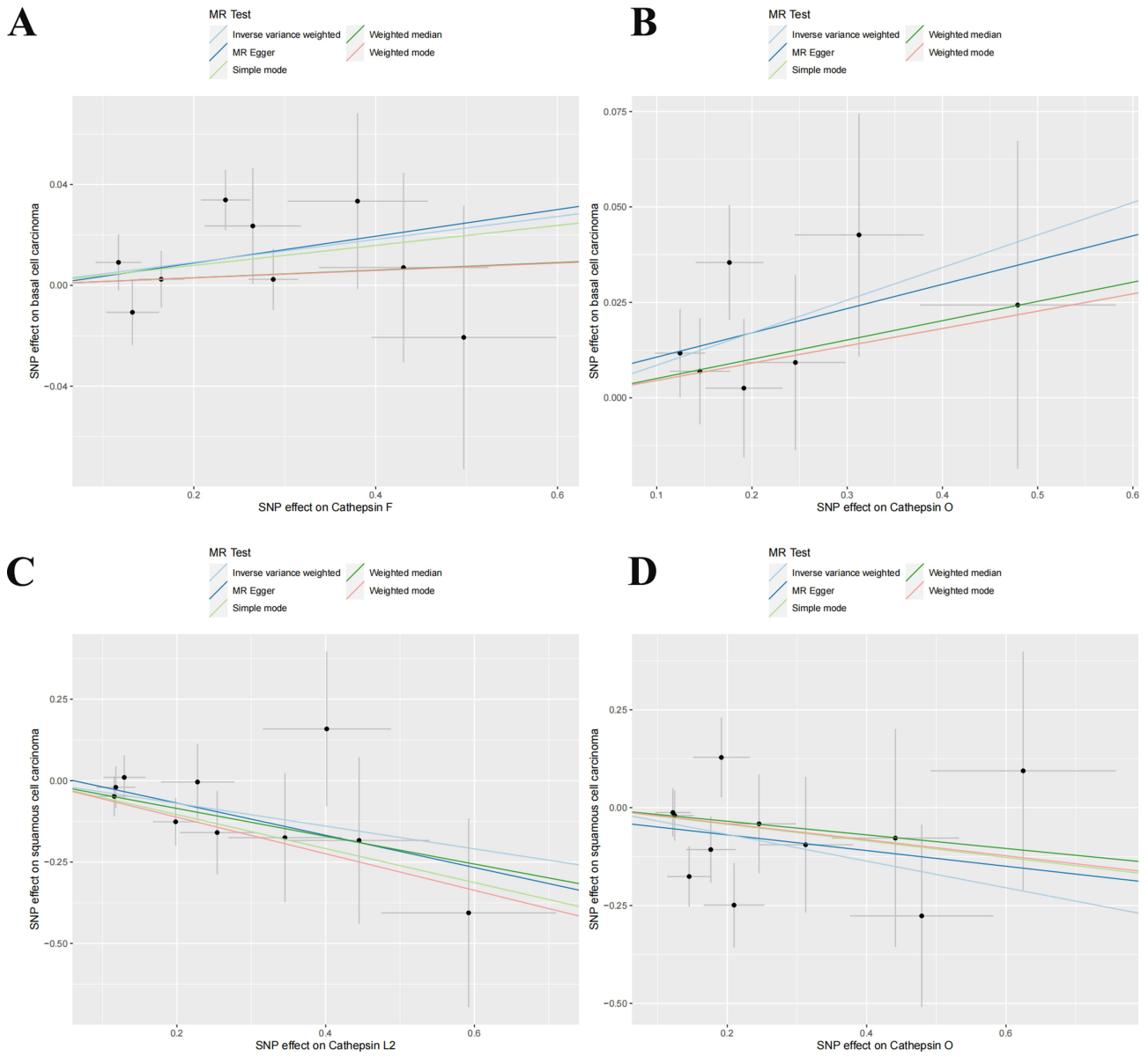

Figure 4 Scatter plot with forward causality in MR. (**A**) Cat-F on BCC. (**B**) Cat-O on BCC (**C**) Cat-L2 on SCC. (**D**) Cat-O on SCC.

BCC, with Cat-F (32 genes) and Cat-O (7 genes) being particularly notable Additionally, 18 genes were identified as inhibiting Cats in SCC, including Cat-L2 (10 genes) and Cat-O (8 genes). These genes with varying levels of expression may influence the progression and development of both BCC and SCC (Supplementary Table 7).

Functional Enrichment Analysis of Genes

To explore the functional and pathway differences between these two groups of genes, we performed KEGG pathway analysis and GO enrichment analysis(Figure 6). KEGG pathway analysis found that genes related to the promotion of BCC formation in Cats-F and Cat-O were mainly enriched in pathways related to lysosomes, apoptotic signaling and phosphonate and phosphonate metabolism related pathways. However, genes related to the inhibition of SCC formation by Cat-L2 and Cat-O were mainly enriched in pathways such as Adipocytokine signaling pathway, AMPK signaling pathway, and Ubiquinone terpenoid-quinone biosynthesis. GO analysis further showed that Cats-F and Cat-O related genes were mainly enriched with lipid binding, phosphatidylinositol binding, and GTPase binding, while Cats-L2 and Cat-O related genes were mainly enriched with anion binding, biotin carboxylase activity and C-methyltransferase activity.

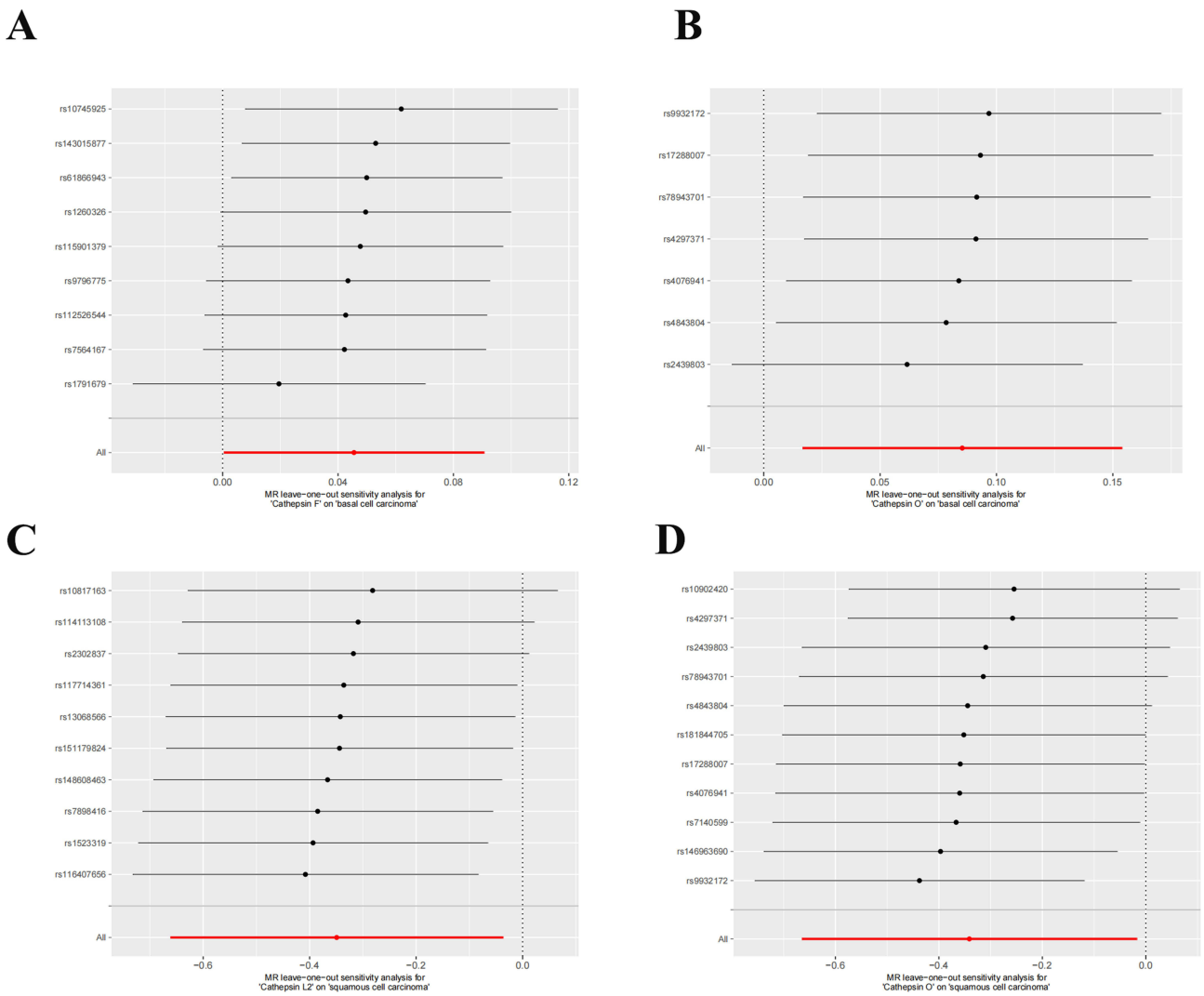

Figure 5 Leave-one-out sensitivity analysis with forward causality in MR. (**A**) Cat-F on BCC. (**B**) Cat-O on BCC (**C**) Cat-L2 on SCC. (**D**) Cat-O on SCC.

Drug Sensitivity Analysis

To explore the drug sensitivity of genes loci related to Cats and skin cancers, we conducted drug sensitivity analyses using the GDSC database. We found that 39 genes were associated with Cats that promote skin cancer, including Cat-F (32 genes) and Cat-O (7 genes), while 18 were associated with Cats that promote skin cancer, including Cat-L2 (10 genes) and Cat-O (8 genes). This suggests that these differentially expressed genes are closely associated with the development and progression of skin cancer. In genes related with Cat-F and Cat-O that promote BCC, the expression of CTSF, SSH3, ARHGAP32, DRAM1, RIN1, NRBP1 and KRTCARP3 is positively correlated with sensitivity to PI-103, BMS345541, BX-912, CX-5461, GSK2126458, Ispinesib Mesylate, PHA-793887, PIK-93, QL-X-138, TG101348, GSK1070916, JW-7-24-1, OSI-027, TPCA-1, Tubastain A, ZSTK474, CP466722, and I-BET-762. While ETFDH, CDH23, PPM1G, ACTN3,CTSW,and CELF2 had a significant negative relation with PI-103, BMS345541, BX-912, GSK2126458, Ispinesib Mesylate, PHA-793887, PIK-93, QL-X-138, TG101348,5-Fluorouracil, XMD14-99,AT-7519,JW-7-24-1,OSI-027,KIN001-102,KIN001-270,TPCA-1,Tubastain A, ZSTK474,CP466722,I-BET-762,TAK-715, Phenformin, WZ3105 (more details in Figure 7A). In genes related with Cat-L2 and Cat-O that inhibit SCC, the expression of ACACB and CELF2 is positively related with sensitivity to Docetaxel, 17-AGG, Bleomycin. However, ACACB and CELF2 had a significant negative relation with THZ-

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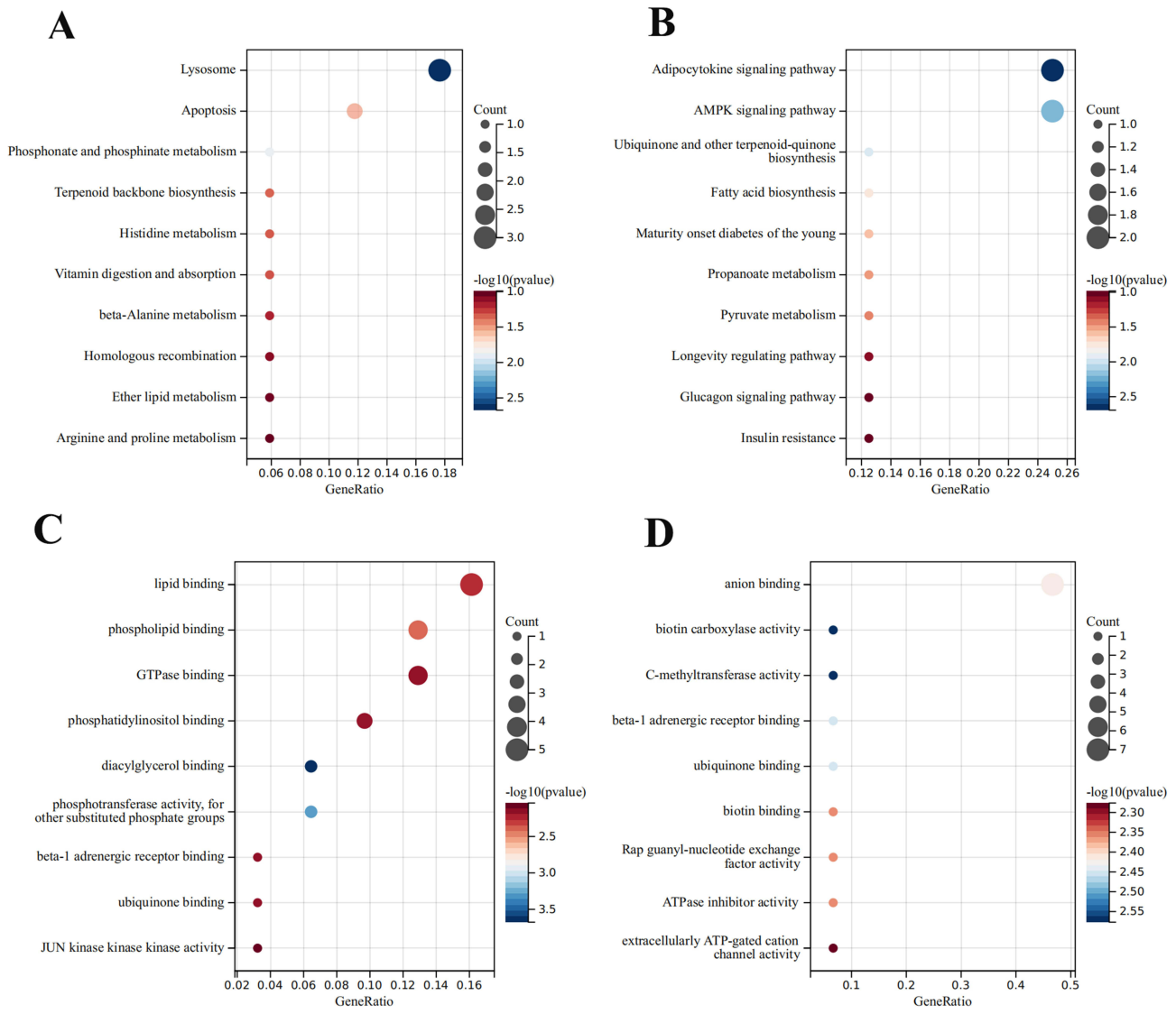

Figure 6 KEGG pathway analysis and GO enrichment analysis for differentially expressed genes located in proximity to genetic loci linked with both Cats (Cat-F, Cats-O and Cat-L2) and skin cancer (BCC and SCC). (**A**) KEGG for genes related to Cats (Cat-F and Cat-O) promoting BCC formation. (**B**) KEGG for genes linked to Cats (Cat-L2 and Cat-O) inhibiting SCC formation. (**C**) GO for genes related to Cats (Cat-F and Cat-O) promoting BCC formation. (**D**) GO for genes linked to Cats (Cat-L2 and Cat-O) preventing SCC formation.

2-49, VNLG/124, KIN001-266,PIK-93,MPS-1-IN-1,Phenformin,THZ-2-102-1,Vorinostat,YM201636,BX-912,KIN001 102, WZ3105,CP466722,NPK76-II-72-1,and OSI-027 (more details in Figure 7B).

Discussion

The critical role of protein hydrolysis in the complex process of tumorigenesis and progression in skin cancer has been extensively studied. Recent research has focused on the impact of different members of the Cat family on skin cancer. By utilizing genetic tools, our study thoroughly investigated the causal relationship between 9 Cat proteins (Cat-B, Cat-E, Cat-F, Cat-G, Cat-H, Cat-L2, Cat-O, Cat-S, and Cat-Z) and the skin cancer (MM, BCC, and SCC). Our results suggest that the various Cats may have distinct effects on the development of these skin cancers. Specifically, we found a potential causal association between Cat-F and Cat-O with BCC, as well as a causal relationship between Cat-L2 and Cat-O with SCC. Elevated levels of Cat-O and Cat-F were related with a higher risk of BCC, while Cat-O and Cat-L2 were linked with a lower risk of SCC. Our research emphasizes the diverse impacts of Cats on different types of skin

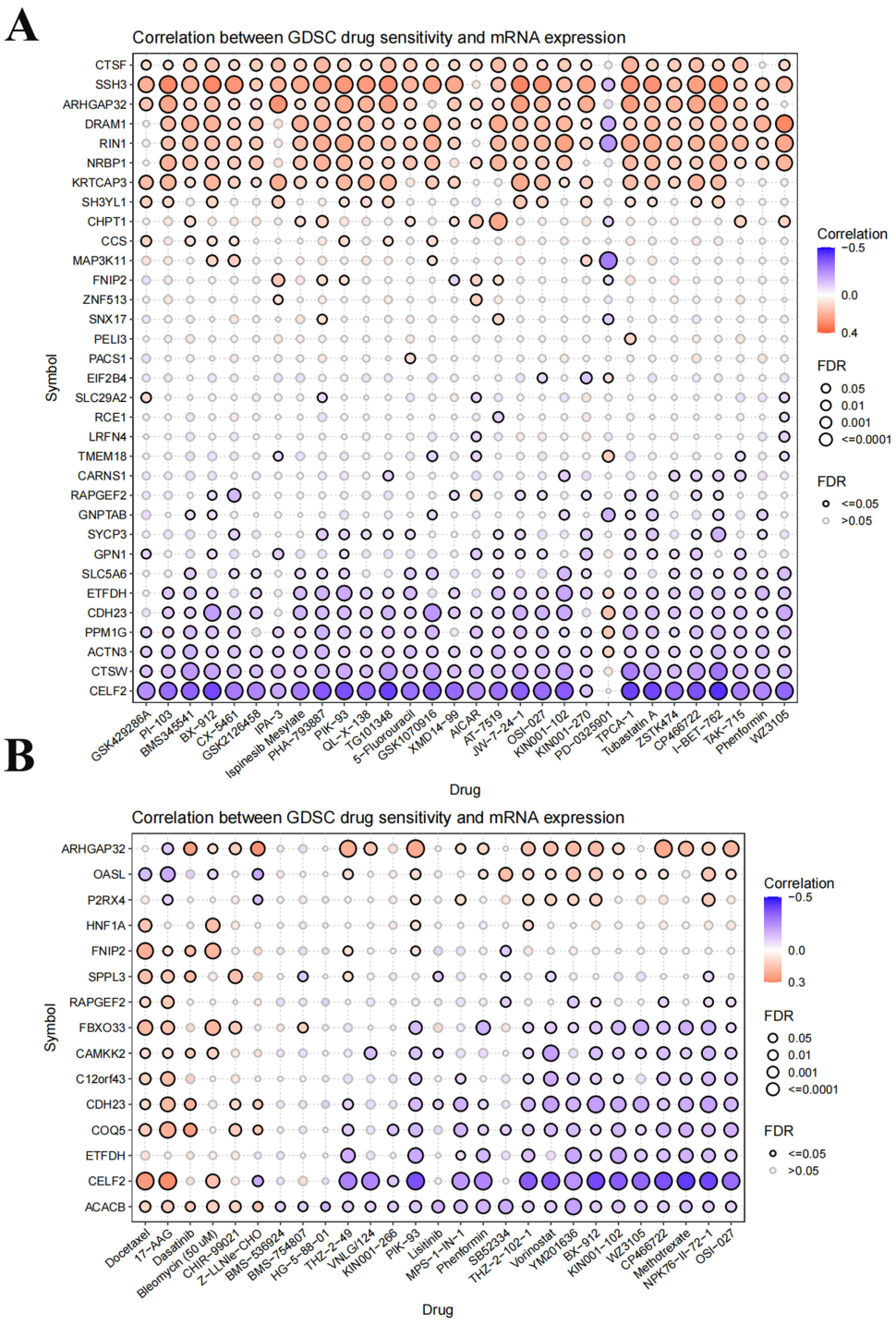

Figure 7 Drug sensitivity analysis for differentially expressed genes located in proximity to genetic loci associated with both Cats (Cat-F,Cat-O,and Cat-L2)and skin cancer (BCC and SCC). (**A**) Drug sensitivity analysis for genes related with Cats (Cat-F and Cat-O) promoting BCC formation. (**B**) Drug sensitivity analysis for genes linked to Cats (Cat-L2 and Cat-O) preventing SCC formation.

cancer. Additionally, we have outlined the molecular mechanisms by which Cats influence the formation of BCC and SCC through the creation of schematic diagrams (Figure 8).

Our research has uncovered a new discovery that suggests Cat-F and Cat-O could potentially increase the risk of BCC. Cat-F, known as a protein hydrolase, which breaks down peptide bonds of enzymes in lysosomes, can be found in a range of lower organisms and tissues.[26,27] Acting as a disease regulator, Cat-F is involved in the development and

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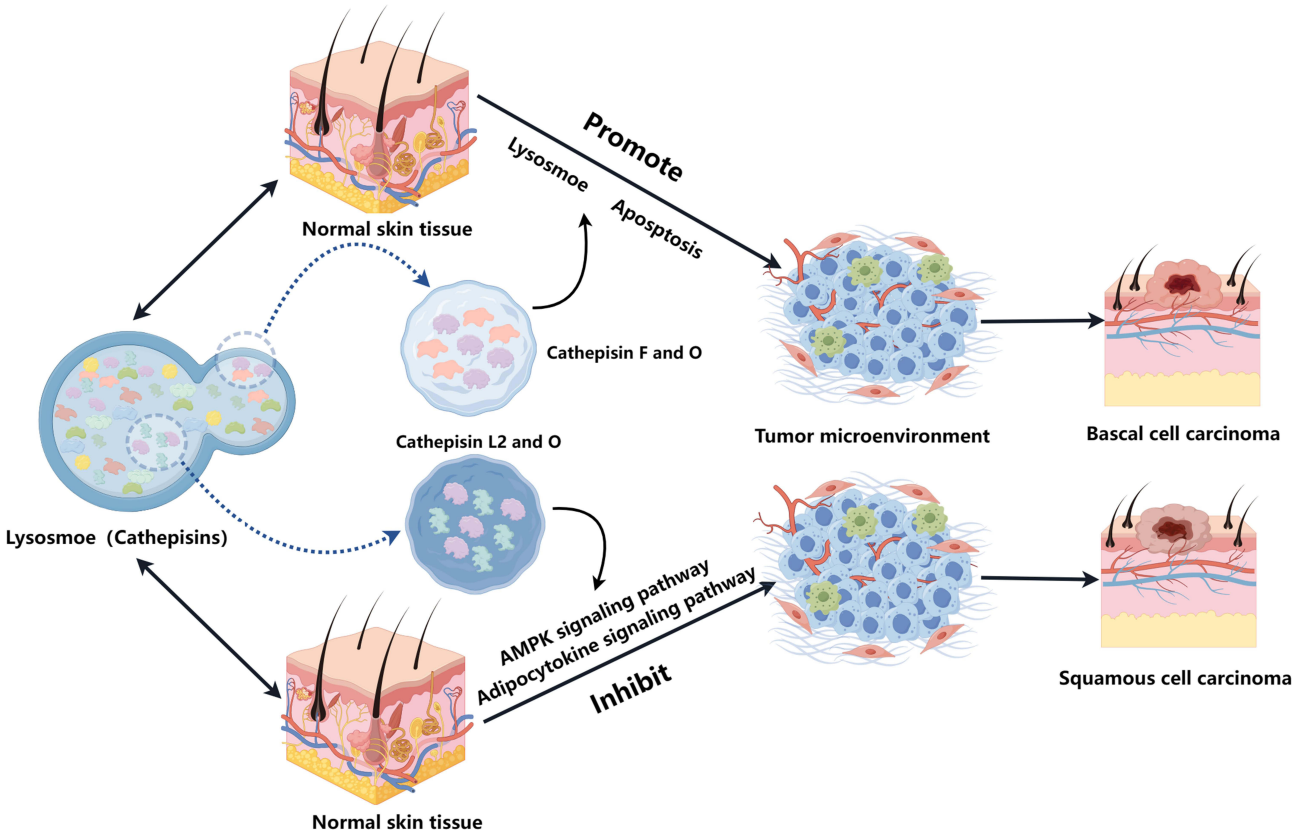

Figure 8 The mechanism of Cats (Cat-F, Cat-O, Cat-L2) and affecting the formation of skin cancer (BCC and SCC) by Figdraw.

advancement of several malignant tumors including gastric cancer,[28] cervical cancer[29] and lung cancer.[30] Previous studies have reported that Cat-F is associated with dermatitis and gives cells the ability to immortalize by binding to Bcl family proteins (Bcl-2 and Bcl-xl),[31] which is one of the reasons why tumor cells continue to proliferate.[32] Nonetheless, the research on the correlation between Cat-F and BCC is scarce. The mechanism by which Cat-F influences BCC risk is likely multifactorial, involving changes in genetic regulation, cellular signaling, immune response, and possibly DNA repair mechanisms. If Cat-F has a direct role in promoting basal cell proliferation, inhibiting tumor suppressors, or modulating immune surveillance, it could contribute to an increased risk of BCC. To clarify this link, further research into the specific functions of Cat-F in skin cells and its interactions with other molecular pathways is needed. Understanding whether Cat-F acts as an oncogene or tumor suppressor in the context of BCC will be crucial for unraveling its exact role in skin cancer development. Our MR analysis findings will serve as a crucial point of reference for upcoming researchers investigating the link between Cat-F and BCC. In previous observational studies, some Cats have been found to be associated with the development and progression of BCC. Specifically, Cat-B, Cat-K, and Cat-L are highly expressed in BCC.[14,15] Furthermore, in BCC, Cat-K has been shown to potentially enhance the invasive and metastatic capacities of tumor cells by breaking down the extracellular matrix.[15] Our MR analysis did not definitively reveal a causal relationship between Cat-B, Cat-K, and Cat-L and the progression of BCC. Cat-O is a fresh endoprotease that potentially participates in breaking down the external matrix and has received less focus in comparison to other Cats.[33] Cairns and colleagues found that CTSO is implicated in reducing protein levels of BRCA1 and ZNF423 through cysteine protease-mediated degradation, leading to resistance to tamoxifen in estrogen receptor alpha (ERα)-positive breast cancer patients and a poorer prognosis.[34] Our MR analysis indicated that increased levels of Cat-O are related to a higher risk of BCC but a decreased risk of SCC, suggesting that Cat-O may have divergent effects on the development and advancement of different types of skin cancer. BCC primarily arises from the basal cells in the epidermis and is often associated with mutations in pathways like the hedgehog signaling pathway, particularly in response to UV radiation.[35]

SCC originates from squamous cells and tends to be more aggressive. Its development is often linked to mutations in the p53 tumor suppressor gene and other UV-induced genetic alterations, alongside immune evasion.[36] If Cat-O influences different signaling pathways or genetic loci related to the development of BCC and SCC, it could have opposing effects on these cancers. For instance, it might upregulate or downregulate different molecular mechanisms that either promote or inhibit the development of BCC versus SCC. This underscores the importance of studying Cat-O in the context of skin cancer, but further comprehensive research is necessary to unravel its intricate mechanisms.

By MR analysis, we discovered that Cat-L2 can decrease susceptibility to BCC. Cat-L2, also known as Cat-V, acts as a versatile endopeptidase primarily involved in the maturation of major histocompatibility complex (MHC) class II molecules and the release of antigenic peptides. It also participates in the cleavage of intracellular and extracellular substrates, as well as the turnover of elastinogen fibers.[37] Many studies have indicated that abnormalities in Cat-L expression are linked with the progression of a variety of diseases. in endometrial cancer, high levels of Cat-L2 expression have shown a positive correlation with the expression of growth regulators like MYBL2, cyclin B1, Ki-67, and p21/WAF, potentially influencing the advancement of the disease.[38] In bladder cancer, Cat-L2 has been shown to promote tumor cell proliferation by activating NF-kB.[39] Moreover, in lung cancer, Cat-L2 is involved in cleaving adhesion molecules like fibronectin, E-calmodulin, and N-calmodulin, which inhibit T-lymphocyte activity and stimulate metastasis and tumor cell spread.[40] In skin-related diseases, Cat-L has been observed to be highly expressed in chronic inflammatory skin conditions and skin cancers, particularly psoriasis, atopic eczema, and SCC.[18] Our study suggests that Cat-L2 reduced the risk of SCC, which is contrary to previous findings and may be due to biased results from confounding factors. This indicates a potentially intricate link between Cat-L2 and SCC, underscoring the need for further comprehensive investigations into the underlying mechanisms.

The molecular mechanisms underlying the MR results were further elucidated through KEGG pathway analysis of Cat-O and Cat-L related genes. These genes may inhibit the SCC formation by modulating the AMPK signaling pathway. Activation of AMPK signaling was found to effectively suppress the downstream pathway (such as AKT/mTOR signaling), thereby restricting the proliferation of SCC cells.[41] Our analysis also revealed an association between the Adipocytokine signaling pathway and BCC formation, consistent with previous findings. Vuletic et al[42] demonstrated that the leptin receptor (LEPR), a receptor for adipokines, is linked to the proliferative index and degree of differentiation of SCC. The expression level of LEPR can serve as a predictor of SCC malignancy. Additionally, adipokines such as Aspirin (ASP) and meteorin-like peptide (METRNL) have been implicated in the invasive process of SCC. Our study further suggested that Cat-F and Cat-O may promote BCC progression by modulating lysosomal pathways and apoptotic signaling. However, our findings are subject to further exploration and their potential therapeutic significance seems to be underestimated.

In the pharmacological treatment of BCC and SCC, we also conducted an in-depth exploration. We identified a group of genes - CTSF (rs1791679), SSH3 (rs1791679), ARHGAP32 (rs2439803), DRAM1 (rs10745925), RINI (rs1791679), and NRBP1 (rs1260326) - located near genetic loci associated with Cat-O and Cat-F, which showed a positive association with BCC formation. The expression of these genes was found to be positively correlated with sensitivity to 5-Fluorouracil. A clinical study involving 29 BCC patients treated with 5% 5-FU cream twice daily for 12 weeks led to a histological cure rate of 90%, with a short mean treatment duration of 10.5 weeks. Patients reported minimal discomfort, scarring, and mostly mild erythema.[43] Furthermore, combining 5-Fluorouracil with other drugs like imiquimod and Tretinoin has shown promise in preventing BCC development.[42] We also identified another group of genes, ARHGAP32 (rs2439803), OASL (rs148608463), and P2RX4(rs148608463), which are located near genetic loci associated with Cat-L2 and Cat-O. Vorinostat, a HDAC inhibitor, showed a positive correlation between these genes and their sensitivity. In SCC, Vorinostat hinders cell proliferation by inhibiting the expression of cyclins D1, D2, E, and A, while also inducing apoptosis by downregulating mTOR, AKT, and ERK signaling pathways.[44] Due to its potent anti-SCC activity, Vorinostat is considered a promising candidate for SCC treatment. Through drug sensitivity analyses, we have identified potentially effective drug candidates for BCC and SCC.

The study presents several strengths. Firstly, although lots of previous research have shown that Cats are closely associated with skin cancer development, progression, and prognosis, however, these existing studies are observational and their results are susceptible to confounding factors, leading to potentially unreliable results. In this study, the association between Cats and skin cancer was explored for the first time using a large-scale GWAS dataset and MR analysis. Secondly, to exclude bias and heterogeneity during the study, a range of methods such as Cochrane's

Q statistics, simple mode, weighted mode and median mode were adopted. In addition, using IVW and MR-Egger regression intercept term tests, we tested and excluded horizontal pleiotropy. Therefore, our results have greater reliability relative to observational studies. Nevertheless, there are a few limitations in our study. Firstly, our analysis was restricted to individuals of European descent, which may restrict the generalizability of our findings to other ethnic populations due to varying genetic characteristics among different groups. Secondly, our results were primarily derived from statistical analyses, necessitating further experimental and clinical validation.

Conclusion

Our MR study provides new evidence for a causal relationship between Cats and skin cancer. Increased levels of Cat-F and Cat-O were genetically predicted to be linked with a higher risk of BCC, whereas increased levels of Cat-L2 and Cat-O were protective factors for SCC. Through bioinformatics analysis, we found that it may affect the formation and progression of skin cancer through regulatory pathways such as AMPK signaling pathway and apoptotic pathway. More in-depth studies are necessary to be performed to confirm the causal relationship and potential molecular mechanisms between Cats and skin cancer, providing valuable directions for the prevention and treatment of skin cancer.

Data Sharing Statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed at the corresponding author.

Ethics Statements

According to Article 32 of the Ethical Review Measures for Life Science and Medical Research Involving Human Beings of the People's Republic of China, the data used in this study will not cause any form of harm to human beings, nor will it touch sensitive personal privacy or trade secrets, so the ethical review can be exempted. In addition, the database used in this study was publicly available and legally available.

Acknowledgments

We sincerely thank all individuals and researchers for making the genome-wide summary statistics from the respective GWAS publicly accessible.

Funding

There is no funding to support.

Disclosure

The authors declare no conflicts of interest in this work.

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