Nischarin expression may have differing roles in male and female melanoma patients

Marija Ostojić (vidosavljevic.marija@gmail.com)
Institut za onkologiju i radiologiju Srbije Odeljenje za eksperimentalnu onkologiju
https://orcid.org/0000-0003-3489-3559

Marko Jevrić
Institut za onkologiju i radiologiju Srbije

Olivera Mitrović-Ajtić
Institute for Medical Research

Miljana Tanić
Institut za onkologiju i radiologiju Srbije Odeljenje za eksperimentalnu onkologiju

Milena Čavić
Institut za onkologiju i radiologiju Srbije Odeljenje za eksperimentalnu onkologiju

Tatjana Srdić-Rajić
Institut za onkologiju i radiologiju Srbije Odeljenje za eksperimentalnu onkologiju

Jelena Grahovac
Institut za onkologiju i radiologiju Srbije Odeljenje za eksperimentalnu onkologiju
https://orcid.org/0000-0003-1430-8682

Research Article

Keywords: nischarin, melanoma, microdeletions, hyper-methylation, sex-related disparities

Posted Date: August 4th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1576440/v2

Version of Record: A version of this preprint was published at Journal of Molecular Medicine on June 29th, 2023. See the published version at https://doi.org/10.1007/s00109-023-02339-y.

Abstract

Due to the development of resistance to previously effective therapies, there is a constant need for novel treatment modalities for metastatic melanoma. Nischarin (NISCH) is a druggable scaffolding protein reported as a tumor suppressor and a positive prognostic marker in breast and ovarian cancers through regulation of cancer cell survival, motility and invasion. The aim of this study was to examine the expression and potential role of nischarin in melanoma. We found that nischarin expression was decreased in melanoma tissues compared to the uninvolved skin, and this was attributed to the presence of microdeletions and hyper-methylation of the *NISCH* promoter in the tumor tissue. In addition to the previously reported cytoplasmic and membranous localization, we observed nischarin in the nuclei in melanoma patients' tissues. *NISCH* expression in primary melanoma had favorable prognostic value for female patients, but, unexpectedly, high *NISCH* expression predicted worse prognosis for males. Gene set enrichment analysis suggested significant sex-related disparities in predicted association of *NISCH* with several signaling pathways, as well as with different tumor immune infiltrate composition in male and female patients. Taken together, our results imply that nischarin may have a role in melanoma progression, but that fine-tuning of the pathways it regulates is sex-dependent.

Introduction

Malignant melanoma is the deadliest form of skin cancer, with increasing incidence in both men and women [1]. Annually, about 1.7% of all newly diagnosed primary malignant cancer cases and approximately 0.6% of all cancer deaths worldwide are due to the cutaneous melanoma [2]. If diagnosed and treated early, melanoma is curable in 93% of patients [1], but the patients with distant metastases have a dire prognosis. In the last decade, advancements in targeted and immunotherapies, particularly MAP kinase pathway inhibitors (BRAF, MEK) and immune checkpoint blockers (CTLA-4, PD-1), have dramatically increased median survival for patients with non-resectable metastatic disease from 6–10 months to 24 months [3]. Unfortunately, significant proportion of patients does not respond to treatment, or develop resistance and eventually relapse [4]. In addition, many melanoma patients worldwide do not have access to first-line innovative therapeutics [5]. Therefore, there is an urgent need for both novel therapeutic targets and affordable treatment strategies for melanoma.

Nischarin (NISCH, Imidazoline receptor 1 (IR1), Imidazoline receptor antisera selected (IRAS)) is a scaffolding protein, first cloned from the human hippocampal library twenty years ago [6]. It was first identified as a cytosolic protein anchored to the inner layer of the plasma membrane, bound to alpha 5 beta 1 (α5β1) integrin and involved in cytoskeletal organization [7]. Nischarin is also of great interest as a target in drug discovery as a non-adrenergic imidazoline receptor and has been shown to couple to several transduction pathways including cAMP pathway, G protein coupled receptor signaling, MAPKs ERK and c-Jun and nitric oxide [8]. Data from the Human Protein Atlas (HPA) repository indicate that the NISCH is expressed in almost all human tissues, with the highest expression in cerebellum, adrenal gland, bronchus, rectum, gallbladder, heart muscle, and skin [9]. In cancer, *NISCH* has been defined as a tumor suppressor, with decreased expression in breast and ovarian cancer compared to the healthy tissue [10,

11]. *NISCH* gene is located at the 3p21.1, in the region described as a putative tumor suppressor cluster [12]. The tumor suppressive role of NISCH stems from its ability to regulate cytoskeletal signaling through interaction with the integrin α5 and formation of focal adhesions and invadopodia [7, 13–15]. This ultimately has an impact on cancer cell survival, motility and invasion. Nischarin has also been shown to alter cancer cell energy metabolism through LKB1-AMPK-mTOR axis [16]. As two major obstacles in melanoma treatment – invasive potential and metabolic plasticity – are potentially linked to NISCH, NISCH is a great target for investigation in the context of melanoma progression and treatment. Importantly, several Food and Drug Administration (FDA) approved NISCH agonists are currently in clinical use [8] which might enable quick integration into melanoma patients treatment options.

Nischarin expression and role in melanoma have not been investigated to date. This study aimed to assess *NISCH* mRNA and protein expression, transcriptional regulation, *NISCH* prognostic value, as well as its potential role in melanoma progression, by examining publicly available datasets and melanoma patient samples, and taking into account sex-related differences.

Materials And Methods

Detailed methods are available in Supplemental Material.

Results

NISCH expression during melanoma progression

We first examined *NISCH* mRNA expression in three publicly available datasets containing samples of normal skin, nevi and primary and metastatic melanoma tissue. Primary melanoma tumor tissue expressed significantly less *NISCH* mRNA than nevi or normal skin; while decrease in expression in nevi compared to the skin was significant in GSE3189 [17] and presented as a trend in GSE46517 [18] (Fig. 1a). There was no difference in *NISCH* mRNA expression between the primary tumor and the metastatic sites when the metastases samples were grouped into lymph node metastases and other metastases (Fig. 1b). To validate these findings, we examined melanoma tissues from patients operated at our institution (IORS cohort). While there was no difference in expression between primary tumors and other metastatic sites, lymph node metastases had significantly lower NISCH expression compared to primary melanoma samples (Fig. 1c).

Next, we examined the protein expression and localization of NISCH in the samples described in the HPA and in our patient samples. In the HPA that uses the HPA023189 antibody, NISCH was detected in healthy skin (Fig. 2a), with high expression in melanocytes, keratinocytes and Langerhans cells and medium expression in fibroblasts. The localization of the protein was described as cytoplasmic and membranous, which corresponds to the so far described functions of the protein. In the melanoma samples in the HPA, NISCH was described as weakly expressed in the 50% of the samples, and intriguingly in 75% of the samples its localization was described as nuclear (Fig. 2b). In IORS primary melanoma patient samples

NISCH could be observed both in the dermis and the epidermis, while in the dermal compartment it was present both in the cytoplasm and the nuclei (Fig. 2c). Vimentin and H&E staining were performed in consecutive sections to label the mesenchymal compartment. NISCH protein expression could also be detected in the lymph node and distant metastases, and the amount of protein corresponded to the level of mRNA (Fig. 2d). In the metastatic samples too, the staining could be observed both in the cytoplasm and the nucleus (Fig. 2e). These results imply that in melanoma NISCH could have so far unreported role in the nucleus.

Regulation of *NISCH* gene expression in melanoma

As we observed a decrease in *NISCH* mRNA expression in primary tumors compared to nevi in both IORS patient cohort and publicly available datasets, as well as possible decrease in expression in lymph node metastases compared to primary tumors, we next examined the mechanisms of *NISCH* down-regulation in melanoma. In breast cancer, loss of *NISCH* expression was reported as a consequence of a loss of heterozygosity (LOH) on the chromosomal locus 3p21 and microdeletions in introns 2, 3 and 6 of the *NISCH* gene [14]. In ovarian cancer, silencing was reported to be mainly due to the *NISCH* promoter hyper-methylation [10]. In melanoma, we first analyzed *NISCH* gene copy-number in TCGA SKCM and DFCI [19] datasets. These datasets contain only metastatic melanoma samples for copy-number analysis. NISCH shallow deletions were detected in around 20% of TCGA SKCM and 15% of DFCI samples. Samples in which shallow deletions were detected had significantly lower *NISCH* expression (Fig. 3a, p=0.0001 for TCGA SKCM, p=0.0183 for DFCI) and samples with the gain of gene copies had higher *NISCH* expression compared to the diploid samples (Fig. 3a, p=0.0004 for TCGA SKCM). In our patient cohort, we detected microdeletions in the *NISCH* gene promoter (ΔKCT below −0.65) in 30% of lymph node samples and in 40% of other metastases samples (Figure 3b). Collectively, these results imply that in metastatic melanoma microdeletions may be a common mechanism for decreased *NISCH* mRNA expression.

Next, we analyzed the methylation status of the *NISCH* promoter in the TCGA SKCM and GSE86355 [20] datasets. In the TCGA SKCM dataset, the methylation status did not significantly differ in melanoma compared to the normal skin samples, but in GSE86355 dataset there was a trend of increased methylation with tumor progression and in metastatic melanoma samples *NISCH* promoter was significantly more methylated than in nevi (Fig. 3c, ANOVA p=0.0254, Dunett's multiple comparisons test p=0.0405). In the IORS patient cohort, we detected hyper-methylation of the *NISCH* promoter in 3/8 primary tumor samples compared to nevi, 8/10 lymph node metastases, and all 5 of other metastases samples (Fig. 3b and 3d). In conclusion, both microdeletions in the *NISCH* gene and hyper-methylation of the *NISCH* promoter are possible mechanisms of *NISCH* mRNA decrease in the lymph nodes and the distant metastatic sites in melanoma. In the primary tumor samples only hyper-methylation was present.

In addition, we looked into the *NISCH* gene mutation status in the TCGA and DFCI melanoma samples and observed that 3.86% of all TCGA SKCM samples (2/76 primary, 12/287 metastatic melanoma) and 5.79% DFCI samples (7/121 metastatic melanoma) had a mutation present. Majority of *NISCH* mutations

in the TCGA SKCM dataset (for which data on the sex was available) were detected in males – two samples in the primary tumor group that were mutated were both males, and 9 out of 12 metastatic samples with the detected mutation (Fig. 3e). These sex-related differences in *NISCH* mutational status are important to be noted, as they may have functional implications.

Sex differences in the prognostic value of *NISCH* expression in melanoma

As expression of *NISCH* was decreased in melanoma samples compared to normal skin and nevi, we sought to determine whether *NISCH* levels had any association with clinical or pathological patient data. Unlike in breast and ovarian cancers where *NISCH* mRNA and protein expression decreased with grade [10, 11], there was no difference in *NISCH* mRNA levels in tumors classified by Clark's level, Breslow depth, mitotic rate or cancer stage in the TCGA SKCM and GSE46517 datasets (not shown). It should be noted that these results must be interpreted with caution due to the small number of samples and their uneven distribution.

Using the best *NISCH* expression cut-off to yield maximal difference with regard to survival between the high and low expression groups, *NISCH* was, surprisingly, an unfavorable prognostic marker in melanoma (HR=2.05, 95% CI of ratio 0.8629-4.871, p=0.0496) (Fig. 4a). As this was in contrast with so far reported prognostic value for *NISCH* in breast and ovarian cancers [10, 14], we re-analyzed the prognostic value in males and females separately. We found that *NISCH* was a favorable prognostic marker in female melanoma patients (HR=0.3116, 95% CI of ratio 0.09046-1.073, p= 0.0446), and unfavorable in male melanoma patients (HR=3.738, 95% CI of ratio1.112-12.57, p= 0.0019) (Fig. 4b). This difference was not due to the different expression of *NISCH* in males and females, as the levels of *NISCH* mRNA were comparable (Fig. 4c). Analysis of the additional dataset GSE46517 confirmed that *NISCH* prognostic value differed between male and female patients (Supplemental Fig. S1). Taken together, our findings imply that *NISCH* may have a sex-dependent role in melanoma progression and that its role in cancer progression may be tumor-specific.

Nischarin associated gene networks

To examine the observed sex-related disparity in *NISCH* prognostic value in melanoma, we performed gene set enrichment analysis (GSEA) using Hallmark, KEGG and Reactome gene sets in males and females separately. The majority of signaling pathways involved in regulation of metabolism (mTORC1 signaling, gluconeogenesis, glycolysis, tricarboxylic acid (TCA) cycle, oxidative phosphorylation), cell cycle checkpoints, unfolded protein response and ABC protein transport were significantly negatively associated with increasing *NISCH* expression for both sexes (Fig. 5a). On the other hand, there were signatures that were inversely associated with *NISCH* in males and females. The most prominent differences were in the Hedgehog and WNT signaling pathways and WNT ligand biogenesis and trafficking, which were positively associated with high *NISCH* expression in females and negatively in males. In males, *NISCH* had a negative association with distinct metabolic pathways as well as cell cycle checkpoint and nucleotide excision repair (NER) (Fig. 5b, supplemental table S3). The effect of patient sex on WNT gene family in melanoma was especially notable since majority of WNT genes had strong

correlation with high *NISCH* expression in female patients, but not in males (Fig. 5c). Our analysis implies that *NISCH* has a role in melanoma metabolic plasticity, but that the fine-tuning of the pathways is sex-dependent.

Association of NISCH with Immune Infiltration

It is well documented that anticancer immune response differs in men and women (previously reviewed [21]), and that sex-associated molecular differences in tumor mutation burden in males have an impact on immune response and survival of melanoma patients [22]. To examine the possible impact of the immune cell content on the sex-dependent *NISCH* role in melanoma, we downloaded Infiltration Estimation file for all TCGA SKCM primary tumors and formed four groups based on the *NISCH* expression and sex to match groups used in the survival analysis (Supplemental Fig. S2). Six tools that allow inter-sample comparisons of the same cell type (TIMER, CIBERSORT-ABS, EPIC, quanTIseq, xCell, and MCP-counter) come with a set of cell type signatures ranging from 6 immune cell types to 64 immune and non-immune cell types. Out of all the examined immune infiltrate cell types, males with high *NISCH* expression – that had the worst prognosis – had significantly higher levels of tumor infiltrating B cells than the males with low *NISCH* expression according to 4 out of 5 methods with this cell type listed (Fig. 6a and Supplemental Fig. S2). Memory B cell counts, analyzed only in CIBERSORT-ABS and xCell, were higher in $NISCH^{high}$ males compared to $NISCH^{low}$ males, but also higher than in females independently of *NISCH* levels according to CIBERSORT-ABS (Figure 6b). Almost no differences in immune cell infiltration were noted between high and low *NISCH* female tumor samples. It has been reported that female melanoma patients may have a more diverse B cell response, and that the presence of distinct tumor-infiltrating B cell subsets may be associated with patient outcome [23]. However, functional phenotype and B cell receptor repertoire profile need to be validated by traditional tumor imaging techniques before drawing final conclusions. Taken together, our results imply that there is a sex-dependent difference in active signaling pathways and a subset of the immune infiltrate that associate with *NISCH* expression in melanoma.

Discussion

Immune checkpoint inhibitor therapy and targeted BRAF and MEK inhibitors have revolutionized melanoma treatment in the past decade, but only a subset of patients have complete and durable response to therapy. In search for new therapeutic targets, we examined the expression, regulation and potential role of nischarin in progression of melanoma. NISCH was described as a tumor-suppressive protein for its role in regulation of the cell migration and invasiveness [11, 15]. *NISCH* expression has been shown to decrease with progression in breast and ovarian cancer [10, 11, 24] leading to increased cell migration, tumor growth, and cell survival [11]. Restoring NISCH levels in breast cancer cells reduced tumor growth *in vivo* and cell migration *in vitro* by inhibiting cancer cells ability to attach to the extracellular matrix [15], decreased FAK phosphorylation and prevented ERK activation [14, 25]. Since *ERK* activation is one of the main mechanisms of resistance to BRAF inhibitors [26], we hypothesized that

NISCH may be a good candidate to investigate in metastatic melanoma, given that there are several approved agonists for this receptor.

In this study, we examined *NISCH* expression in publicly available datasets and samples collected at our institution – in normal human skin, nevi, primary and metastatic tumor samples – and found that it is expressed at both mRNA and protein level, and that its expression is decreased in tumors compared to skin and nevi. Surprisingly, unlike in breast and ovarian cancers, where NISCH mRNA and protein expression decreased with grade [10, 11], we did not find any association of *NISCH* mRNA levels in melanoma tumors with clinical or pathological patient data. Opposite of the dominantly cytoplasmic and membranous localization that was reported in breast cancer cells, NISCH was also detectable around and in the nucleus in melanoma cells, similarly to its localization in neurons [27], cells that share common embryonic origin with melanocytes [28]. Nuclear localization of NISCH has not been previously described in cancer, and may imply difference in function in epithelial- and neuroectodermal-derived cells, the aspect that needs further study

Regulation of *NISCH* expression in melanoma was in line with the previously reported mechanisms, indicating presence of microdeletions and hyper-methylation of the *NISCH* promoter in the tumor tissue. *NISCH* promoter hyper-methylation was reported in 30% of breast and 36.7% of ovarian cancer tissues [10, 14]. In lung cancer, *NISCH* gene was also hyper-methylated and inactivated [29]. In our study, *NISCH* promoter hyper-methylation was detected in several primary melanoma samples, but its impact on *NISCH* levels was the most pronounced in metastatic melanoma samples. Both IORS experimental cohort and analysis of the GSE86355 data indicated that in majority of metastatic melanoma samples *NISCH* promoter was hyper-methylated compared to nevi (13/15 and 20/28, respectively), indicating that this is a common occurrence in melanoma. This observation was also in concordance with the study that examined aberrant promoter methylation in a panel of neural crest-derived tumors, where *NISCH* promoter was hyper-methylated in 80% of melanoma samples [30]. Copy number alteration was the other proposed mechanism of NISCH expression regulation in cancer [11]. In our study, microdeletions were present in metastatic samples with evidently lower *NISCH* levels, implying that in metastatic melanoma this may be an important mechanism of *NISCH* downregulation. Microdeletions and hyper-methylation of *NISCH* promoter are not mutually exclusive events since both could be seen in the same patient in IORS cohort. Co-occurrence of different mechanisms of *NISCH* inactivation was also observed in TCGA SKCM dataset (not shown), but also in ovarian cancer where both *NISCH* promoter hyper-methylation and LOH occurred in the same tumor samples [10]. Knowing that malignant melanoma carries one of the highest mutational burdens [31], it is not surprising that *NISCH* mutations are detected in both primary and metastatic melanoma samples in *in silico* analyzed datasets. The majority of *NISCH* mutations in TCGA SKCM dataset were detected in males. This was not unexpected as male melanoma patients have greater mutational burden overall [32], but functional implications of *NISCH* mutations need further examination.

Surprisingly, *NISCH* appeared to be an unfavorable prognostic marker in melanoma, unlike in ovarian cancer where patients with higher *NISCH* expression had better overall survival [10] and in breast cancer where patients with higher *NISCH* expression had increased recurrence-free survival [14]. As this was an

unexpected finding, and bearing in mind that breast and ovarian cancers were examined in females, we analyzed the effects of *NISCH* mRNA expression level in primary melanoma in females and males separately. Strikingly, in females *NISCH* was a favorable prognostic marker, while in males it was unfavorable. Sex differences in cancer driver genes and sex-influenced biomarkers of cancer patient outcome are present across a broad range of tumor types [33], and our findings indicate that tumor-suppressive role that has been reported for *NISCH* may not be universal for female and male patients. In melanoma, women have up to 30% advantage in all aspects of the disease progression, most likely due to underlying biological sex difference in tumor-host interactions [34]. Female melanoma patients also have lower incidence and better survival compared to males (that persists after adjustment for various confounding variables such as stage, body site, age and metastatic status) [35, 36]. We did not observe the difference in the levels of *NISCH* expression in males and females in publicly available datasets or in our cohort, and we hypothesize that tumor-host interactions influence *NISCH* prognostic role. Indeed, in *NISCH* loss of function mutant mice (with leucine-rich repeat (LRR) domain deletion that prevents NISCH interaction with AMPK [16]), male and female mice showed divergent glucose metabolism and sexual dimorphism in insulin sensitivity [37].

Since *NISCH* has been predominantly investigated in female cancer patients, and its effects in males are unknown, we performed GSEA in male and female melanoma samples separately. Our analysis revealed that regardless of sex *NISCH* expression had significant correlation with many metabolic pathways. This is not surprising since it has been shown that *NISCH* plays an important physiological role in metabolic homeostasis [38]. Groups of genes involved in mTORC1 signaling, regulation of glucose metabolism and TCA cycle had a negative correlation with *NISCH* both in females and males. Mammalian target of rapamycin complex 1 (mTORC1) serves as a central regulator of cell metabolism, growth, proliferation, invasion, and survival [39], and has been found to be almost universally activated in melanoma [40]. Regulation of mTORC1 at the signaling level includes its suppression by LKB1 through activation of AMPK [41]. Previous study showed that interaction between LKB1 and NISCH inhibits cell migration and breast tumor progression [42], but more recently NISCH connection to LKB1-AMPK pathway was established in mutant mouse model with NISCH deletion. *NISCH* mutant mice showed significantly decreased activity of AMPK [37], suggesting that NISCH is important regulator of AMPK-mTOR signaling pathway. Many metabolic processes differ in males and females, with great contribution from the X-linked metabolic genes (reviewed in [43]), and NISCH association with regulation of cell metabolism may have different effects in male and female patients. Of importance, there were distinct signaling pathways with opposite association dependent on the patient sex. NER and cell cycle checkpoint regulation were negatively associated with higher *NISCH* expression in males, but not in females. As NER is responsible for repair of UV-induced DNA induced damage this may contribute to the higher mutation burden frequently observed in male melanoma patients [44]. In females, but not in males *NISCH* was positively associated with WNT signaling, especially with *WNT8B, WNT6, WNT3A*, and *WNT8D* (Fig. 5c). Although it has been previously reported that *WNT6* and *WNT8* both play a critical role in the neural crest induction during embryonic development [45], their role in melanoma remains unclear. On the other hand, elevated nuclear beta catenin and activation of the WNT/beta catenin signaling by WNT3A in melanoma was

associated with reduced proliferation and improved survival [46] and BRAF inhibitor-induced apoptosis was mediated, in part, through WNT3A [47, 48]. This could be one possible explanation for positive association of high NISCH expression with better survival in female patients.

Elevated WNT/beta catenin signaling has also been reported to prevent anti-tumor immunity in melanoma [49] and decreased T cell infiltration [50]. The only significant difference between *NISCH*^{high} and *NISCH*^{low} tumors in males based on our *in silico* analysis of the tumor immune infiltrate was in the increased presence of memory B cells in *NISCH*^{high} tumors. We did not observe any significant differences in immune infiltrate in female patient tumors based on *NISCH* expression. This result needs further validation by immunohistochemistry and analysis of the localization and functional type of the B cell infiltrate which was beyond the scope of the present study.

In conclusion, our study brings two important and novel findings that open new avenues for investigation of the nischarin role in cancer. First, we report a previously unreported localization of nischarin in the cell nucleus in melanoma tissues. Second, we highlight the role of sex in melanoma biology and its impact on nischarin role in melanoma progression, aspect not previously addressed in mechanistic studies of nischarin in cancer. This finding questions the tumor suppressive role of nischarin and warrants examination of its prognostic role in other cancer types since NISCH was previously investigated only in female cancers. Limitations of this study include a relatively small cohort of samples and the fact that it is was performed as a single-center study, thus validation of the obtained results in a multi-center setting is needed.

Declarations

Funding

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Agreement No. 451-03-68/2022-14/200043 and 451-03-68/2022-14/200015). JG is supported by Horizon 2020 MSC grant agreement No. 891135.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Marija Ostojić, Marko Jevrić, Tatjana Srdić-Rajić and Jelena Grahovac contributed to the study conception and design. Material preparation, data collection and analysis were performed by Marija Ostojić, Marko Jevrić, Olivera Mitrović-Ajtić, Miljana Tanić, Milena Čavić, and Jelena Grahovac. Tatjana Srdić-Rajić and Jelena Grahovac secured the acquisition of funds for the study. The first draft of the manuscript was written by Marija Ostojić and Jelena Grahovac, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

The datasets analyzed during the current study are available at the following web links:

1) NCBI GEO repository: GSE3189, GSE46517, GSE86355

2) Broad Institute GDAC Firehose

3) cBioPortal: TCGA SKCM, DFCI dataset

4) The Human Protein Atlas: skin, melanoma samples

5) Mexpress

Ethics approval and

The study was approved by the Ethics Committee of the Institute for Oncology and Radiology of Serbia (Approval No5549-01, from 11.12.2017) and was performed in accordance with the Helsinki Declaration of 1975, as revised in 2013.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent to publish

Not applicable.

Acknowledgements

The results shown here are in part based upon data generated by the TCGA Research Network: https://www.cancer.gov/tcga.

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A (2021) Cancer Statistics, 2021. CA Cancer J Clin 71:7–33. https://doi.org/10.3322/caac.21654
2. Sung H, Ferlay J, Siegel RL, et al (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71:209–249. https://doi.org/10.3322/caac.21660
3. Ugurel S, Ro J, Ascierto PA, et al (2020) Survival of patients with advanced metastatic melanoma : The impact of MAP kinase pathway inhibition and immune checkpoint inhibition - Update 2019. Eur J Cancer 130:126–138. https://doi.org/10.1016/j.ejca.2020.02.021

4. Mukherjee N, Schwan J V., Fujita M, et al (2015) Alternative Treatments for Melanoma: Targeting BCL-2 Family Members to De-Bulk and Kill Cancer Stem Cells. J Invest Dermatol 135:2155–2161. https://doi.org/10.1038/jid.2015.145
5. Kandolf Sekulovic L, Peris K, Hauschild A, et al (2017) More than 5000 patients with metastatic melanoma in Europe per year do not have access to recommended first-line innovative treatments. Eur J Cancer 75:313–322. https://doi.org/10.1016/j.ejca.2017.01.012
6. Piletz JE, Ivanov TR, Sharp JD, et al (2000) Imidazoline receptor antisera-selected (IRAS) cDNA: Cloning and characterization. DNA Cell Biol 19:319–329. https://doi.org/10.1089/10445490050043290
7. Alahari SK, Lee JW, Juliano RL (2000) Nischarin, a novel protein that interacts with the integrin α5 subunit and inhibits cell migration. J Cell Biol 151:1141–1154. https://doi.org/10.1083/jcb.151.6.1141
8. Bousquet P, Hudson A, García-Sevilla JA, Li JX (2020) Imidazoline receptor system: The past, the present, and the future. Pharmacol Rev 72:50–79. https://doi.org/10.1124/pr.118.016311
9. Uhlén M, Fagerberg L, Hallström BM, et al (2015) Proteomics. Tissue-based map of the human proteome. Science 347:. https://doi.org/10.1126/SCIENCE.1260419
10. Li J, He X, Dong R, et al (2015) Frequent Loss of NISCH Promotes Tumor Proliferation and Invasion in Ovarian Cancer via Inhibiting the FAK Signal Pathway. Mol Cancer Ther 14:1202–1212. https://doi.org/10.1158/1535-7163.mct-14-0911
11. Maziveyi M, Alahari SK (2015) Breast cancer tumor suppressors: A special emphasis on novel protein nischarin. Cancer Res 75:4252–4259. https://doi.org/10.1158/0008-5472.CAN-15-1395
12. Loginov VI, Bazov I V., Khodyrev DS, et al (2008) Human chromosome 3P regions of putative tumor-suppressor genes in renal, breast, and ovarian carcinomas. Russ J Genet 44:209–214. https://doi.org/10.1134/s1022795408020142
13. Ding Y, Zhang R, Zhang K, et al (2013) Nischarin Is Differentially Expressed in Rat Brain and Regulates Neuronal Migration. PLoS One 8:3–9. https://doi.org/10.1371/journal.pone.0054563
14. Baranwal S, Wang Y, Rathinam R, et al (2011) Molecular characterization of the tumor-suppressive function of nischarin in breast cancer. J Natl Cancer Inst 103:1513–1528. https://doi.org/10.1093/jnci/djr350
15. Maziveyi M, Dong S, Baranwal S, Alahari SK (2018) Nischarin regulates focal adhesion and Invadopodia formation in breast cancer cells. Mol Cancer 17:1–11. https://doi.org/10.1186/s12943-018-0764-6
16. Dong S, Baranwal S, Garcia A, et al (2017) Nischarin inhibition alters energy metabolism by activating AMP-activated protein kinase. J Biol Chem 292:16833–16846. https://doi.org/10.1074/jbc.M117.784256
17. Talantov D, Mazumder A, Yu JX, et al (2005) Novel Genes Associated with Malignant Melanoma but not Benign Melanocytic Lesions. Clin Cancer Res 11:7234–7242. https://doi.org/10.1158/1078-0432.CCR-05-0683

18. Kabbarah O, Nogueira C, Feng B, et al (2010) Integrative genome comparison of primary and metastatic melanomas. PLoS One 5:e10770. https://doi.org/10.1371/journal.pone.0010770
19. Liu D, Schilling B, Liu D, et al (2019) Integrative molecular and clinical modeling of clinical outcomes to PD1 blockade in patients with metastatic melanoma. Nat Med 25:1916–1927. https://doi.org/10.1038/s41591-019-0654-5
20. Wouters J, Vizoso M, Martinez-Cardus A, et al (2017) Comprehensive DNA methylation study identifies novel progression-related and prognostic markers for cutaneous melanoma. BMC Med 15:101. https://doi.org/10.1186/s12916-017-0851-3
21. Özdemir BC, Dotto GP (2019) Sex Hormones and Anticancer Immunity. Clin Cancer Res 25:4603–4610. https://doi.org/10.1158/1078-0432.CCR-19-0137
22. Ye Y, Jing Y, Li L, et al (2020) Sex-associated molecular differences for cancer immunotherapy. Nat Commun 11:1–8. https://doi.org/10.1038/s41467-020-15679-x
23. Selitsky SR, Mose LE, Smith CC, et al (2019) Prognostic value of B cells in cutaneous melanoma. Genome Med 11:1–11. https://doi.org/10.1186/s13073-019-0647-5
24. Okpechi SC, Yousefi H, Nguyen K, et al (2022) Role of Nischarin in the pathology of diseases: a special emphasis on breast cancer. Oncogene 41:1079–1086. https://doi.org/10.1038/s41388-021-02150-4
25. Botti G, Cerrone M, Scognamiglio G, et al (2013) Microenvironment and tumor progression of melanoma: New therapeutic prospectives. J Immunotoxicol 10:235–252. https://doi.org/10.3109/1547691X.2012.723767
26. Sullivan RJ, Flaherty KT (2013) Resistance to BRAF-targeted therapy in melanoma. Eur J Cancer 49:1297–1304. https://doi.org/10.1016/j.ejca.2012.11.019
27. Ding Y, Li Y, Lu L, et al (2015) Inhibition of Nischarin Expression Promotes Neurite Outgrowth through Regulation of PAK Activity. PLoS One 10:e0144948. https://doi.org/10.1371/journal.pone.0144948
28. Yaar M, Park HY (2012) Melanocytes: A window into the nervous system. J Invest Dermatol 132:835–845. https://doi.org/10.1038/jid.2011.386
29. Hoque MO, Kim MS, Ostrow KL, et al (2008) Genome-wide promoter analysis uncovers portions of the cancer methylome. Cancer Res 68:2661–2670. https://doi.org/10.1158/0008-5472.CAN-07-5913
30. La Torre A, Muscarella LA, Parrella P, et al (2012) Aberrant genes promoter methylation in neural crest-derived tumors. Int J Biol Markers 27:. https://doi.org/10.5301/JBM.2012.9766
31. Alexandrov LB, Nik-Zainal S, Wedge DC, et al (2013) Signatures of mutational processes in human cancer. Nature 500:415–421. https://doi.org/10.1038/nature12477
32. Gupta S, Artomov M, Goggins W, et al (2015) Gender Disparity and Mutation Burden in Metastatic Melanoma. JNCI J Natl Cancer Inst 107:221. https://doi.org/10.1093/JNCI/DJV221
33. Li CH, Haider S, Shiah YJ, et al (2018) Sex Differences in Cancer Driver Genes and Biomarkers. Cancer Res 78:5527–5537. https://doi.org/10.1158/0008-5472.CAN-18-0362

34. Joosse A, Collette S, Suciu S, et al (2012) Superior outcome of women with stage I/II cutaneous melanoma: Pooled analysis of four European organisation for research and treatment of cancer phase III trials. J Clin Oncol 30:2240–2247. https://doi.org/10.1200/JCO.2011.38.0584
35. Lasithiotakis K, Leiter U, Meier F, et al (2008) Age and gender are significant independent predictors of survival in primary cutaneous melanoma. Cancer 112:1795–1804. https://doi.org/10.1002/cncr.23359
36. de Vries E, Nijsten TEC, Visser O, et al (2008) Superior survival of females among 10 538 Dutch melanoma patients is independent of Breslow thickness, histologic type and tumor site. Ann Oncol 19:583–589. https://doi.org/10.1093/annonc/mdm498
37. Dong S, Blüher M, Zhang Y, et al (2019) Development of insulin resistance in Nischarin mutant female mice. Int J Obes 43:1046–1057. https://doi.org/10.1038/s41366-018-0241-8
38. Nguyen TH, Yousefi H, Okpechi SC, et al (2022) Nischarin Deletion Reduces Oxidative Metabolism and Overall ATP : A Study Using a Novel NISCH Δ 5-6 Knockout Mouse Model. Int J Mol Sci 23:1374
39. Ciołczyk-Wierzbicka D, Gil D, Zarzycka M, Laidler P (2020) mTOR inhibitor everolimus reduces invasiveness of melanoma cells. Hum Cell 33:88–97. https://doi.org/10.1007/s13577-019-00270-4
40. Damsky W, Micevic G, Meeth K, et al (2015) mTORC1 activation blocks BrafV600E -induced growth-arrest, but is insufficient for melanoma formation. Cancer Cell 27:41–56. https://doi.org/10.1016/j.ccell.2014.11.014.mTORC1
41. Lizcano JM, Göransson O, Toth R, et al (2004) LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1. EMBO J 23:
42. Jain P, Baranwal S, Dong S, et al (2013) Integrin-binding protein nischarin interacts with tumor suppressor liver kinase B1 (LKB1) to regulate cell migration of breast epithelial cells. J Biol Chem 288:15495–15509. https://doi.org/10.1074/jbc.M112.418103
43. Haupt S, Caramia F, Klein SL, et al (2021) Sex disparities matter in cancer development and therapy. Nat Rev Cancer 21:393. https://doi.org/10.1038/S41568-021-00348-Y
44. Kusakabe M, Onishi Y, Tada H, et al (2019) Mechanism and regulation of DNA damage recognition in nucleotide excision repair. Genes Environ 41:1–6. https://doi.org/10.1186/S41021-019-0119-6/FIGURES/3
45. Ji Y, Hao H, Reynolds K, et al (2019) Wnt Signaling in Neural Crest Ontogenesis and Oncogenesis. Cells 8:1173. https://doi.org/10.3390/CELLS8101173
46. Chien AJ, Moore EC, Lonsdorf AS, et al (2009) Activated Wnt/ß-catenin signaling in melanoma is associated with decreased proliferation in patient tumors and a murine melanoma model. Proc Natl Acad Sci U S A 106:1193–1198. https://doi.org/10.1073/PNAS.0811902106
47. Chien AJ, Haydu LE, Biechele TL, et al (2014) Targeted BRAF Inhibition Impacts Survival in Melanoma Patients with High Levels of Wnt/β-Catenin Signaling. PLoS One 9:e94748. https://doi.org/10.1371/JOURNAL.PONE.0094748
48. Conrad WH, Swift RD, Biechele TL, et al (2012) Regulating the response to targeted MEK inhibition in melanoma. Cell cycle 11:3724–3730. https://doi.org/10.4161/CC.21645

49. Spranger S, Bao R, Gajewski TF (2015) Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity. Nature 523:231–235. https://doi.org/10.1038/NATURE14404
50. Li X, Xiang Y, Li F, et al (2019) WNT/β-catenin signaling pathway regulating T cell-inflammation in the tumor microenvironment. Front Immunol 10:2293. https://doi.org/10.3389/FIMMU.2019.02293/BIBTEX

Figures

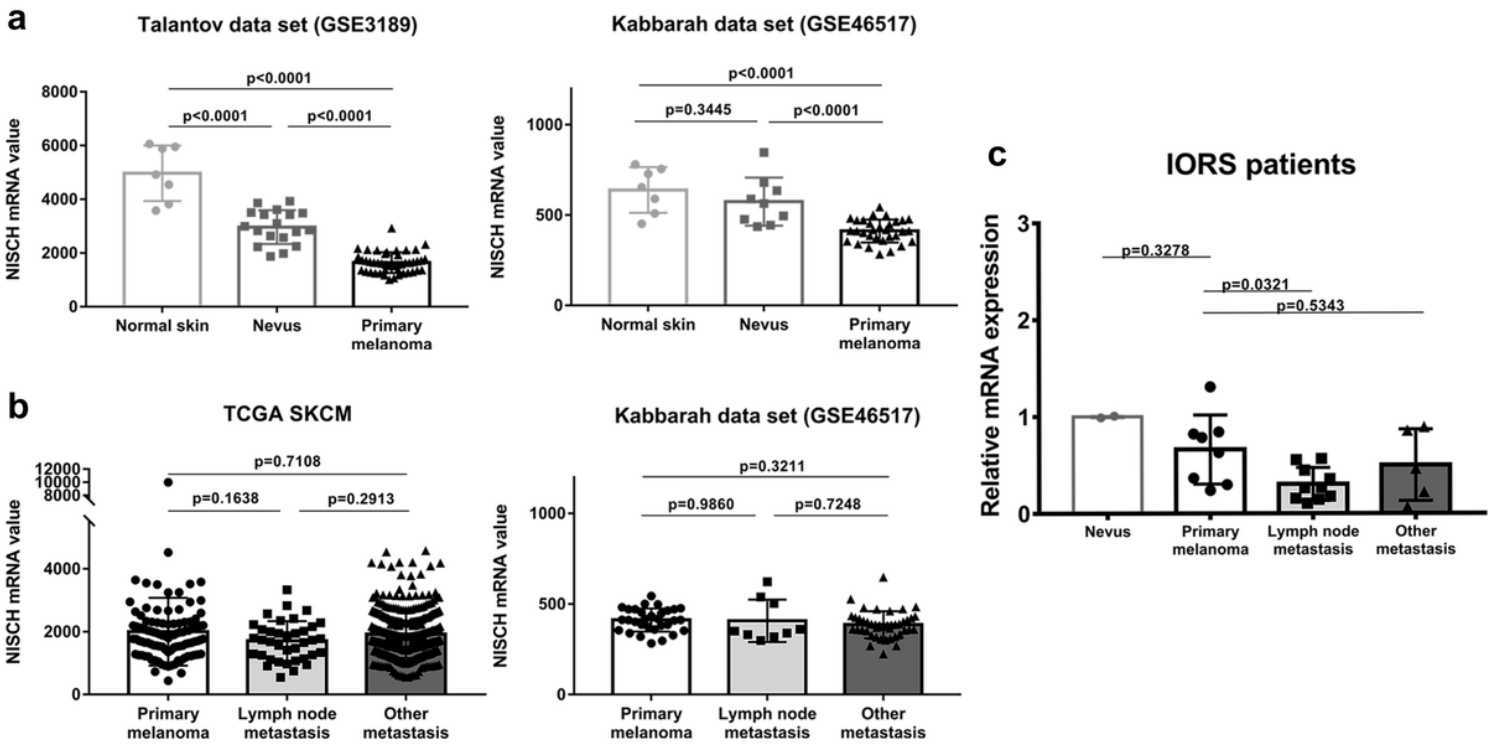


Figure 1

Nischarin mRNA expression in melanoma patients. *NISCH* mRNA expression in (**a**) benign and primary melanoma tissues included in GSE3189 and GSE46517 datasets, and (**b**) primary tumors and metastatic sites from TCGA SKCM and GSE46517 datasets. Data were analyzed by one-way ANOVA with Tukey's multiple comparisons test. (**c**) Relative NISCH mRNA levels in samples of IORS patients measured by qRT-PCR and analyzed using one-way ANOVA with Dunnett's multiple comparisons test

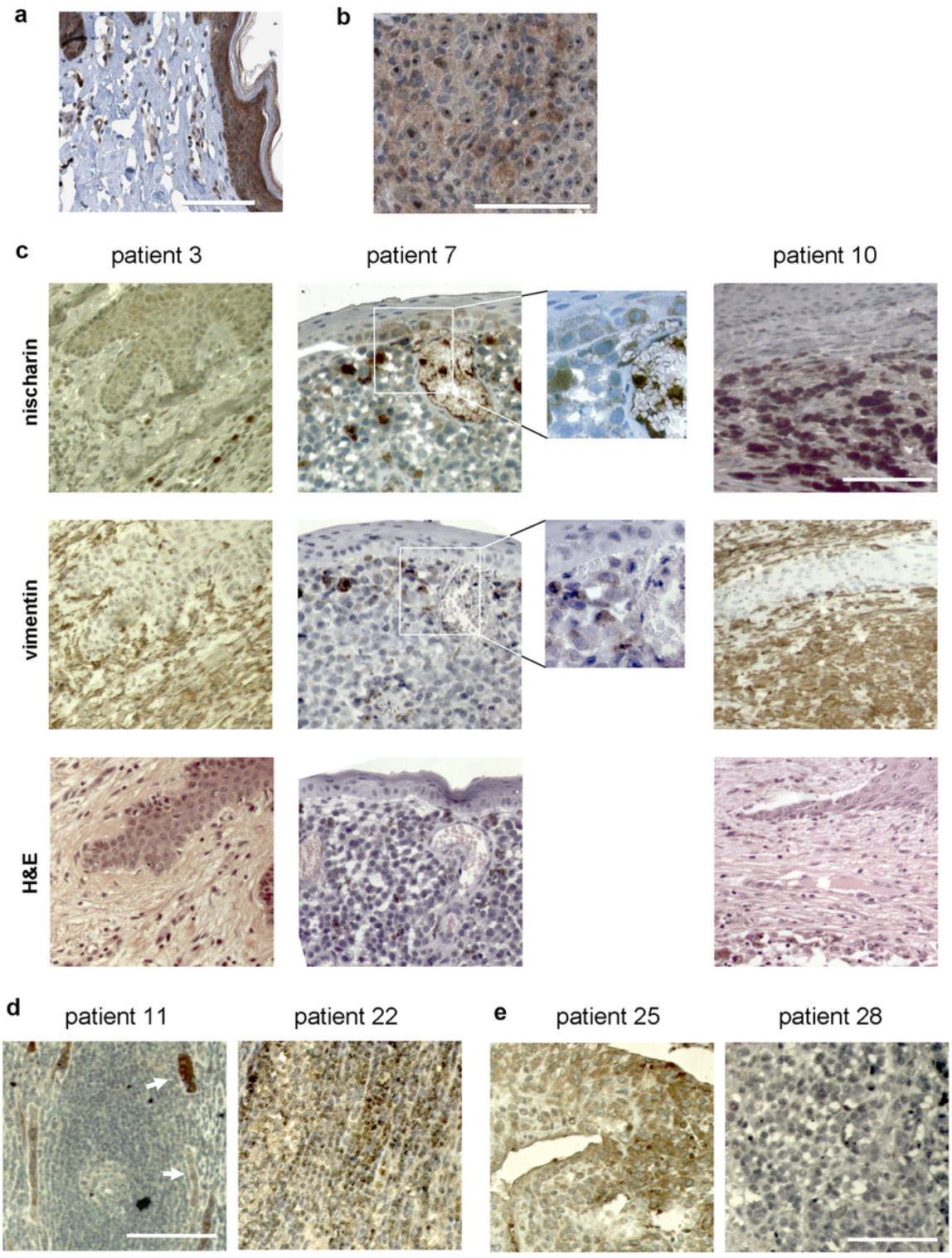


Figure 2

Nischarin protein expression in melanoma patients. Protein expression and localization of NISCH in (**a**) normal skin and (**b**) melanoma sample from the Human Protein Atlas website. (**c**) NISCH, vimentin and H&E staining of primary melanoma samples from IORS cohort. NISCH protein expression was also detected in (**d**) the lymph node and (**e**) distant metastases of IORS patients

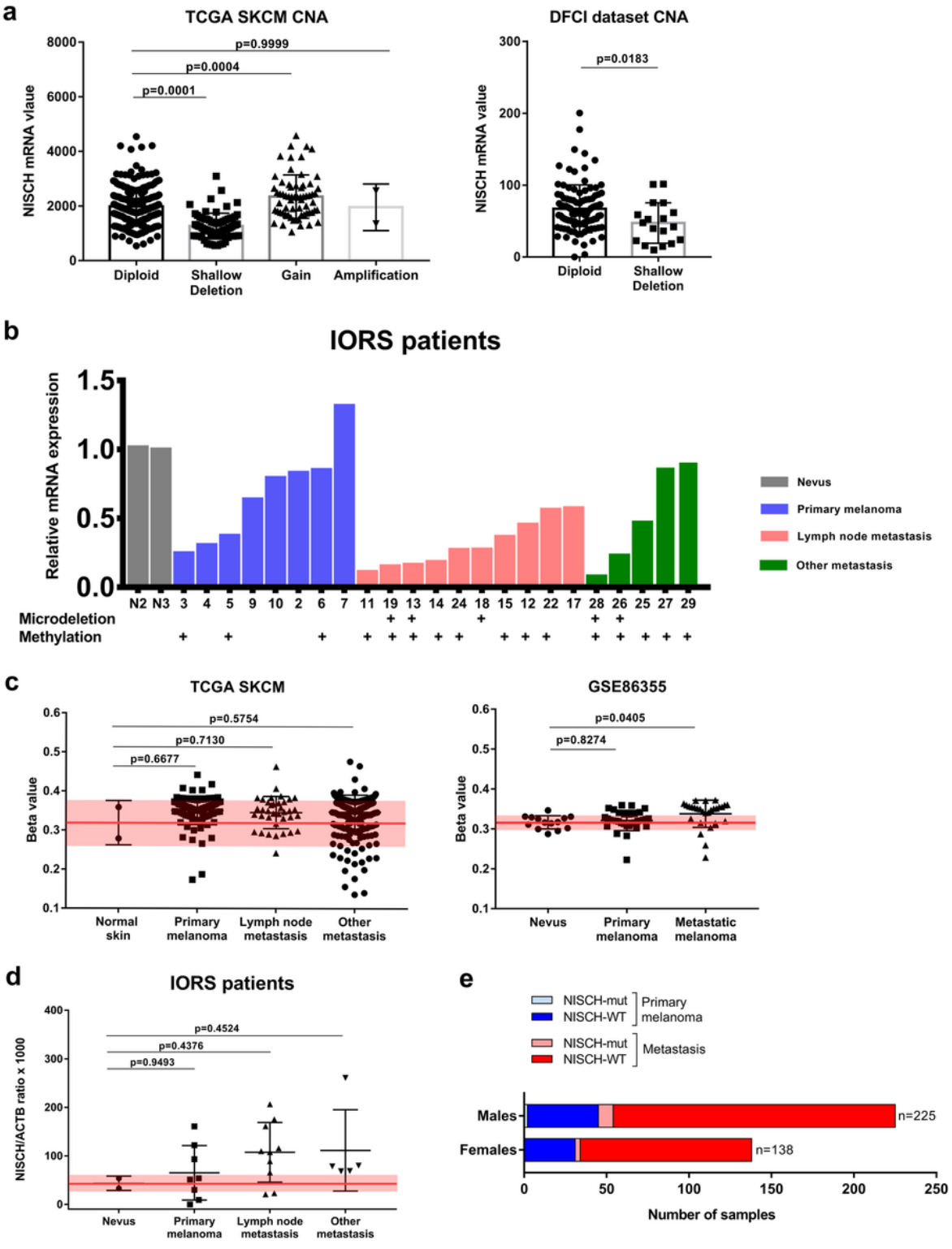


Figure 3

Regulation of nischarin gene expression. (**a**) Somatic copy-number alteration (CNA) analysis of metastatic melanoma samples from TCGA SKCM and DFCI datasets analyzed by one-way ANOVA with Dunnett's multiple comparisons test. (**b**) Levels of NISCH expression in IORS patient samples with

indicated presence of microdeletions and *NISCH* promoter hyper-methylation. (**c**) *NISCH* promoter methylation status in TCGA SKCM and GSE86355 datasets (**d**) and in IORS cohort. All data were analyzed using one-way ANOVA with Dunnett's multiple comparisons test. (**e**) Overview of primary and metastatic melanoma samples from TCGA SKCM dataset with detected *NISCH* mutation

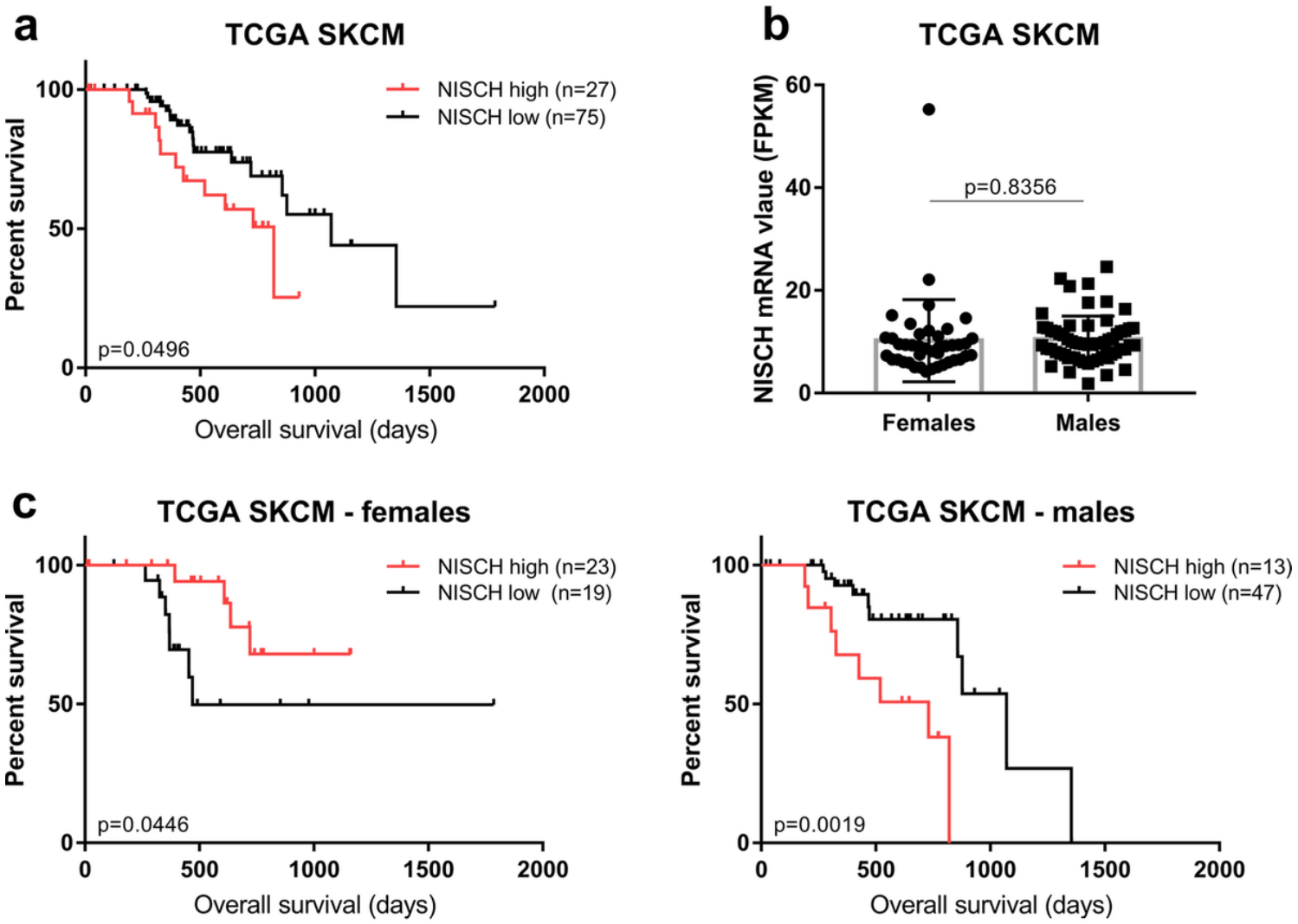
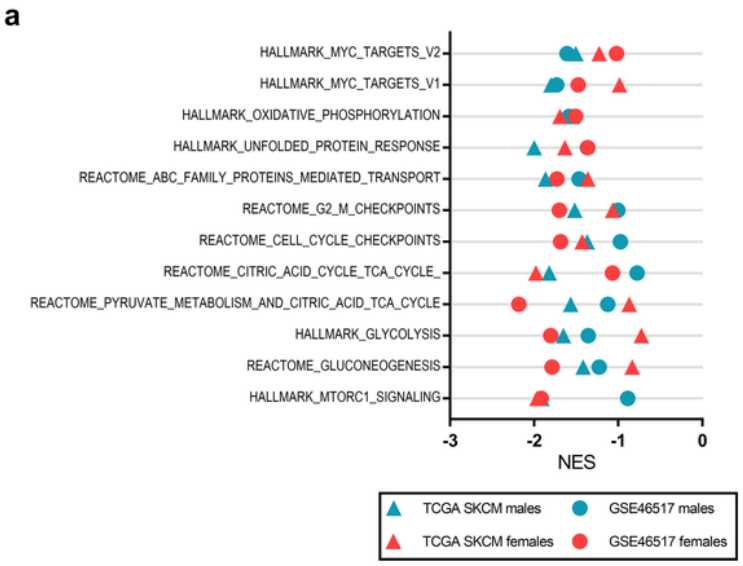


Figure 4

The effect of nischarin mRNA expression on melanoma patients' overall survival. (**a**) TCGA SKCM primary melanoma samples were used for Kaplan-Meier overall survival analysis based on the best NISCH expression cut-off level (11.8 FPKM) according to the Human Protein Atlas. (**b**) These samples were further divided based on patient sex and difference between NISCH mRNA levels in females and males was analyzed using t-test. (**c**) Additional survival analysis was performed for newly formed groups with the best NISCH expression cut-off levels for females (8.23 FPKM) and males (12.7 FPKM)



c

TCGA SKCM	GSEA ranking score	
Gene name	Females	Males
WNT8B	0.903	0.063
WNT6	0.893	0.106
WNT3A	0.877	-0.069
WNT8D	0.875	-0.116
WNT7B	0.846	-0.088
WNT2B	0.829	0.099
WNT7A	0.795	-0.114
WNT10A	0.764	-0.038
WNT9B	0.678	-0.053
WNT3	0.671	-0.076
WNT4	0.454	0.145
WNT1	0.314	-0.184
WNT5B	0.310	0.005
WNT16	0.085	-0.125
WNT11	0.045	-0.040
WNT2	-0.129	-0.164

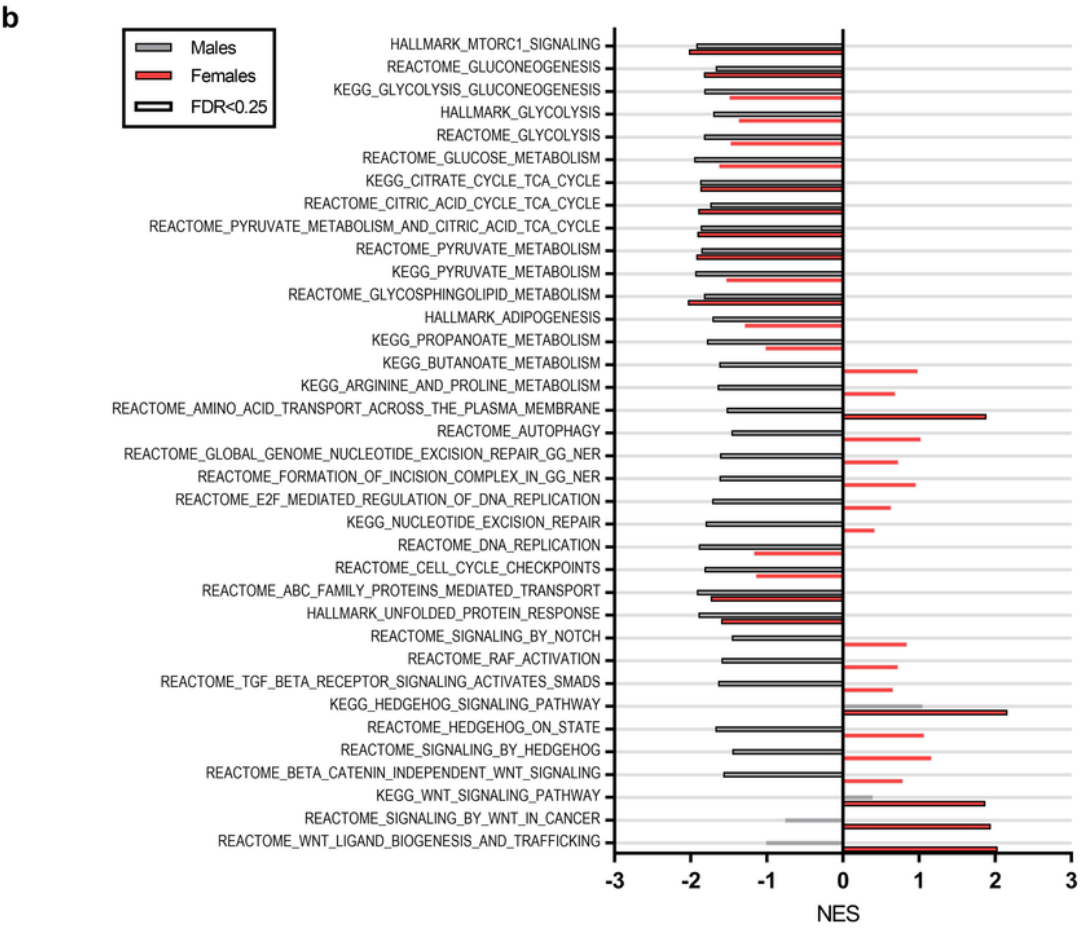


Figure 5

Nischarin associated gene networks in melanoma. (**a**) Gene set enrichment analysis (GSEA) of primary melanoma samples from GSE4651 and TCGA SKCM datasets divided by patient sex. (**b**) Correlation analysis between NISCH and WNT genes based on GSEA ranking score from previous analysis of the TCGA SKCM dataset (Pearson correlation). Red color refers to stronger positive correlation, and blue color

indicates negative correlation. (**c**) GSEA results for TCGA SKCM females and males datasets. NES = normalized enrichment score, FDR = false discovery rate

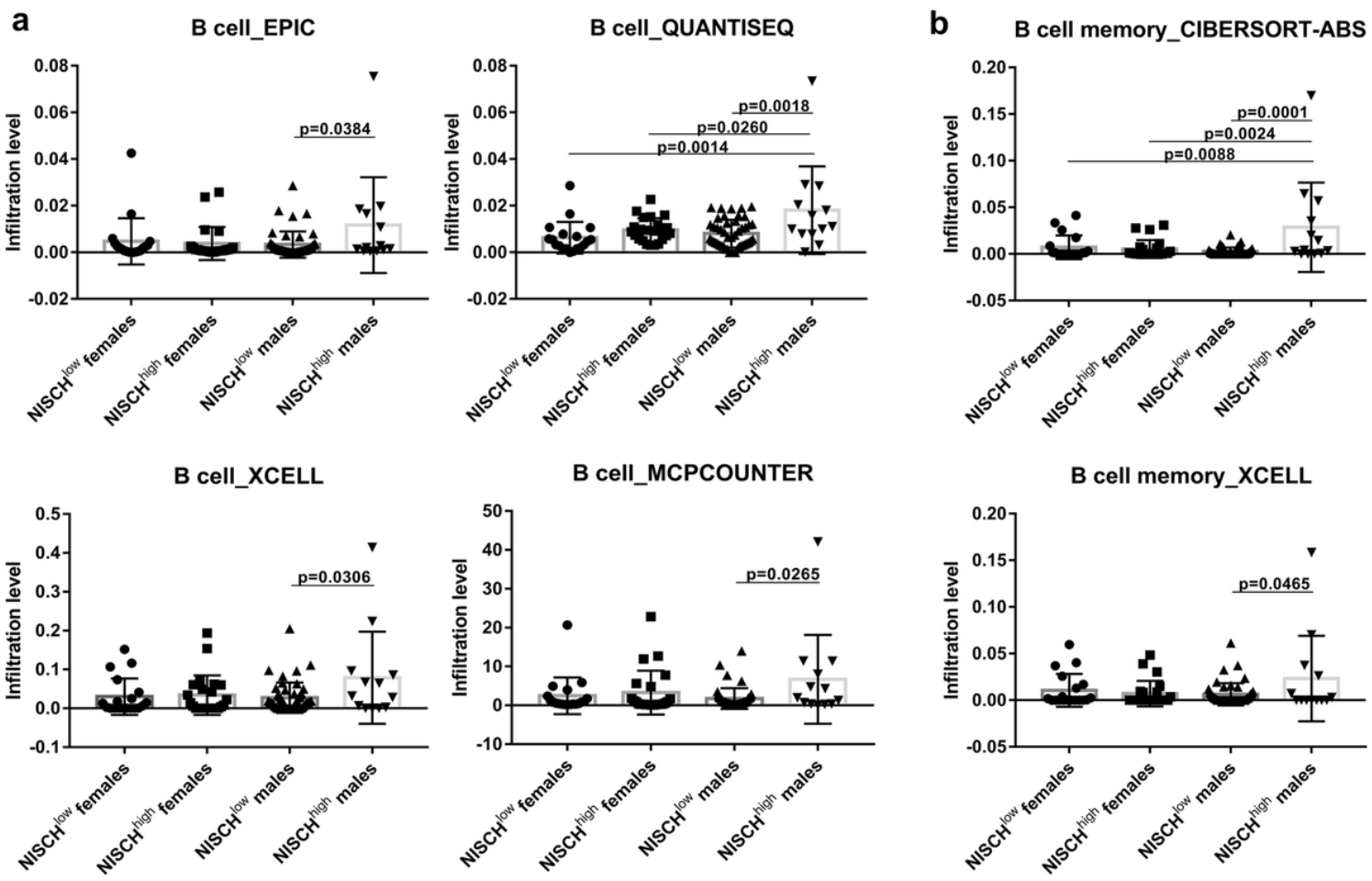


Figure 6

Association of nischarin expression with tumor immune profiles in melanoma samples. Differences in levels of tumor infiltrating (**a**) B cells (according to EPIC, quanTIseq, xCell, and MCP-counter) and (**b**) memory B cell (CIBERSORT-ABS and xCell) in primary melanoma TCGA SKCM samples divided into “high” and “low NISCH” groups for both sexes were examined using one-way ANOVA with Tukey’s multiple comparisons test

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Keymessages.docx

- SupplementaryFigures.pdf
- SupplementaryMaterialsandMethods.pdf
- SupplementaryTables.xlsx