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MicroRNA signatures of cancer risk in kidney transplant patients: insights from the COMETA study

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Abstract

Background Post-transplant malignancies are one of the leading causes of morbidity, mortality and graft failure in kidney transplant recipients (KTRs). While viral infections and immunosuppressive drugs have historically been considered primary causes, the mechanisms underlying post-transplant cancer occurrence remain incompletely understood. Furthermore, predictive cancer biomarkers have yet to be identified in KTRs.

Methods COMETA study is an observational study involving 138 KTRs, and it aims to elucidate the interplay between the immune system and cancer by integrating comprehensive clinical data with high-throughput small-RNA sequencing on the serum of patients with post-kidney transplant malignancies.

Results Our results identified three distinct serum miRNA profiles, respectively, associated with kidney transplantation, pro-oncogenic and onco-protective factors in this unique population. These profiles were also used to create miRNA classifiers, which demonstrated promising predictive performance, especially for tumor-promoting signatures. Notably, miR-210-3p up-regulation, within the cancer-related profile was first found to be associated with non-melanoma skin cancer.

Conclusion These findings could serve as a basis for future research, paving the way for the development of advanced tools for early cancer diagnosis, precise prognosis formulation, and the creation of targeted therapies for KTRs with neoplastic complications.

Keywords Kidney transplant, Non-coding RNA, MiRNA, Cancer

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Background

Over the last two decades, the outcomes of renal transplantation have improved significantly, largely due to advancements in immunosuppressive drugs and protocols [1]. It remains the renal replacement therapy associated with the best health-related quality of life [2, 3]. Therapeutic developments have made acute rejection a rare and frequently manageable cause of graft loss [4]. However, chronic graft rejection and death with a functioning graft have emerged as leading causes of kidney transplant failure globally [5]. Although immunosuppressive therapies have improved short-term graft survival, they also pose long-term challenges, such as increased susceptibility to infections and malignancies, making ongoing monitoring and management essential for improving long-term outcomes in kidney transplant recipients (KTRs) [6, 7]. KTRs represent a population at high risk of developing cancer due to chronic pharmacological immunosuppression, which compromises immune surveillance and promotes oncogenesis. Chronic suppression of T-cell-mediated immunity compromises the body's natural immune surveillance mechanisms, reducing its ability to detect and eliminate emerging malignant cells. This immunosuppressive state creates a permissive environment for oncogenesis, particularly for cancers driven by viral infections and those requiring intact immune responses for control. This condition is further aggravated by the higher prevalence of oncogenic viral infections (such as EBV, HPV, and polyomavirus) and cumulative exposure to genotoxic agents, including immunosuppressive drugs themselves. Several epidemiological studies have demonstrated a two- to four-fold increased risk of cancer in transplant recipients compared to the general population, with particularly elevated incidence rates for non-melanoma skin cancers, post-transplant lymphoproliferative disorders (PTLD), endocrine malignancies and virus-related malignancies [8, 9].

The effectiveness of immune checkpoint inhibitors in several neoplastic diseases, including melanoma, breast, colon, prostate and lung cancers, has reinforced the potential of immunotherapy [10–13]. Recent reports also highlight the use of these drugs to treat malignancies in KTRs contexts [14]. Based on these observations, the understanding of molecular mechanisms that sustain the development of cancer, especially non-melanoma skin cancers, in KTRs is relevant for the prevention, early detection, and timely and personalized treatment.

In recent years, microRNAs (miRNAs) have gained attention as promising non-invasive biomarkers for cancer diagnosis and monitoring [15]. These small RNA molecules regulate gene expression and have been found to reflect not only the presence of cancer, but also its malignant potential and drug resistance [16]. Serum miRNA profiles in cancer patients have been used to identify new biomarkers for various neoplastic conditions [17]. Tumor-derived circulating miRNAs are thought to influence the interaction between cancer cells and the tumor microenvironment, including tumor-specific immune responses. On the other hand, immunosuppressive drugs may regulate the levels of these miRNAs by affecting tumor cells [18]. We conducted the multicentric observational COMETA study. It is based on a large Italian cohort of KTRs and aims to investigate differences in circulating miRNA expression related to the occurrence of post-kidney transplant cancer, considering the duration of exposure to grafts and immunosuppressive regimens. By identifying specific oncogenic and onco-protective miRNA signatures, we aim to clarify the molecular mechanisms that may underlie increased cancer susceptibility in this unique clinical population.

Experimental procedures

Patient recruitment and serum sample collection

COMETA is a retrospective, multicenter and observational study. Participants were enrolled in four University Italian Hospitals: IRCCS Agostino Gemelli in Rome, Fondazione IRRCS Ca' Granda Ospedale Maggiore Policlinico, in Milan, IRCCS Sant'Orsola in Boulogne, and AOU "Luigi Vanvitelli" in Naples. The cohort included pre-kidney transplant (pre-tx) patients, post-kidney transplant (post-tx) patients with cancer and post-tx patients without cancer. Patients under 18 years of age, diagnosed with malignancy within 3 months after kidney transplantation, with prior cancer history and positive for Hepatitis C virus (HCV) and/or Hepatitis B virus (HBV) were excluded.

The study was conducted according to the Declaration of Helsinki and was approved by all local Ethics Committees of the participating centers (Approval nr 0029851/I −2023 by the Ethical Committee of the Coordinating Centre at University of Campania "Luigi Vanvitelli"). All centers followed shared standard operating procedures for blood collection, serum processing, and storage. These protocols were agreed upon prior to patient recruitment and uniformly applied across sites. Clinical and demographic data were collected using a unified database with standardized clinical definitions, ensuring the harmonization of data acquisition across centers. For post-transplant patients with cancer, serum samples were collected during routine follow-up with a cancer diagnosis. Cancer treatment and surgical or dermatological excision had occurred after the sample collection. After obtaining written informed consent from all participants, demographic and clinical data (Table 1; Fig. 1) were collected and catalogued in a dedicated database.

Serum samples were obtained by collecting whole blood from KTRs, and then standard centrifugation at

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Table 1 Demographic and clinical characteristics of the studied cohort of ktrs

Patients characteristics (n = 138)	Post-Tx without cancer (n = 77)	Post-Tx with cancer (n = 30)	Pre-tx (n = 31)
Age (years ± SD)	56.01 ± 12.69	62.4 ± 9.57	55.26 ± 10.74
Gender			
F (n = 41)	27 (35.06%)	7 (23.33%)	7 (22.58%)
M (n = 97)	50 (64.94%)	23 (76.67%)	24 (77.42%)
Race/ethnicity			
Caucasian (n = 136)	75 (97.40%)	30 (100%)	31 (100%)
Asian (n = 2)	2 (2.60%)	–	–
Cancer type			
Solid (n = 30)		CSCC 12 (40.0%)	
		BCC 8 (26.7%)	
		BC 3 (10.0%)	
		RCC 2 (6.7%)	
		SCC 2 (6.7%)	
		CRC 1 (3.3%)	
		PCa 1 (3.3%)	
		PTC 1 (3.3%)	
Single kidney trasplant event	71 (92.21%)	26 (86.67%)	
Multiple kidney transplant events	6 (7.79%)	4 (13.33%)	
Stage			
Stage I	–	23 (76.67%)	
Stage II	–	7 (23.33%)	
CDK etiology			
APDK	13 (16.88%)	6 (20%)	
CN	1 (1.30%)	–	
DM	2 (2.60%)	–	
DMT1-NS	–	1 (3.33%)	
ESKD	3 (3.90%)	5 (16.67%)	
ESKD/Alport	–	1 (3.33%)	
GE	10 (12.99%)	–	
GN	17 (22.08%)	4 (13.33%)	
IgAN	2 (2.60%)	1 (3.33%)	
MN	–	1 (3.33%)	
NAS	6 (7.79%)	3 (10%)	
CAKUT	4 (5.19%)	1 (3.33%)	
P/I	3 (3.90%)	1 (3.33%)	
PDK	3 (3.90%)	3 (10%)	
PN	–	1 (3.33%)	
SD	3 (3.90%)	–	
SG	–	1 (3.33%)	
TIN	–	1 (3.33%)	
U	10 (12.99%)	–	
CNI			
CsA	21 (27.27%)	7 (23.33%)	
FK506	53 (68.83%)	20 (66.67%)	
CsA /FK506	3 (3.90%)	3 (10%)	
mTORi	–		

Table 1 (continued)

Patients characteristics (n = 138)	Post-Tx without cancer (n = 77)	Post-Tx with cancer (n = 30)	Pre-tx (n = 31)
Everolimus	10 (12.99%)	3 (10%)	7 (22.58%)
Rapamycin/Everolimus	5 (6.49%)	2 (6.67%)	1 (3.23%)
MMF			
Mycophenolate	42 (54.55%)	22 (73.33%)	
Everolimus/Mycophenolate	–	1 (3.33%)	
Graft rejection			
Yes	10 (12.99%)	4 (13.33%)	
No	67 (87.01%)	26 (86.67%)	

ADPKD: Autosomal dominant PKD, BC: Breast cancer, BCC: Basal cell carcinoma, CAKUT: Congenital anomalies of the kidney and urinary tract., CDK: Cyclin-dependent kinases, CN: Collagenosic nephropathy, CNI: Calcineurin inhibitors, CsA: Cyclosporin A, CRC: Colon adenocarcinoma, CSCC: Cutaneous squamous sell sarcinoma, DM: diabetes mellitus, DMT1-NS: Nephrotic syndrome in diabetes mellitus type 1, ESKD: End-stage kidney disease, FK506: Tacrolimus, GE: genetic, GN: Glomerulonephritis, IgAN: IgA nephropathy, MN: Membranous nephropathy, MMF: Micophenolate mofetil, mTORi: mTOR inhibitor NAS: Nephoangiosclerosis, NAS: Nephroangiosclerosis, PCa: Prostate cancer, PDK: Polycystic kidney disease, P/I: pyelonephritis-interstitial nephritis, PN: partial nephrectomY, PTC: Papillary thyroid carcinoma, RCC: Renal cell carcinoma, SCC: Spinocellular carcinoma, SD: Symptomatic dialysis, SG: Segmental glomerulosclerosis, TIN: Tubulointerstitial nephritis, U: uncertain.
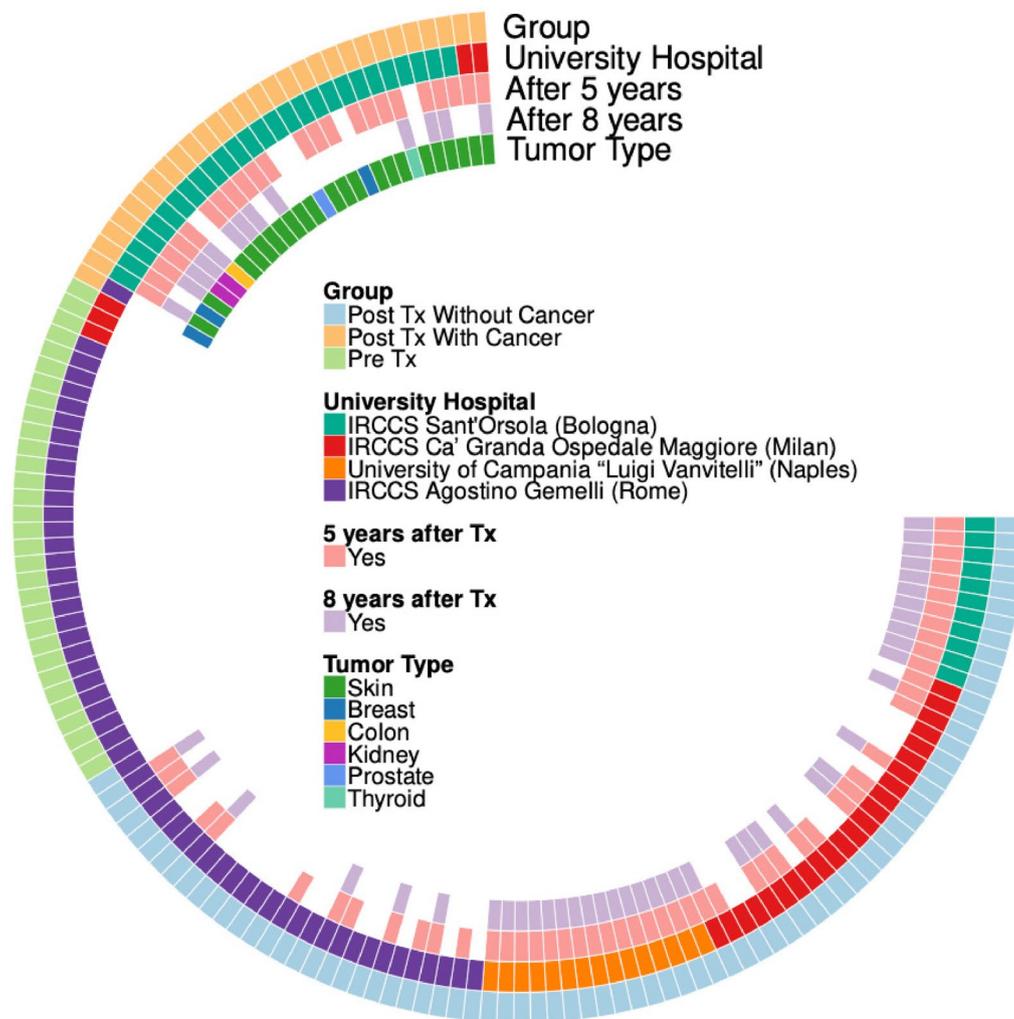

Fig. 1 Patient cohort. The *circos* plot shows the analyzed KTRs population. The samples were annotated according to the belonging group (I track), the originating centre (II track), the sampling time after the kidney transplant (III and IV track) and the cancer type for the samples belonging to the group of post-tx patients with cancer (V track)

room temperature. Aliquots of 500 μl serum samples were stored at – 80 °C in each recruiting center within the COMETA network, and later transported on dry ice to the biobank of Biogem Research Institute (Ariano Irpino, Italy) for miRNA extraction and sequencing.

RNA extraction and sequencing

Total RNA extraction was performed according to the protocol provided by Plasma/Serum RNA Purification Midi Kit (Cat. 56100- Norgene). Purified RNA samples were collected and quantified using RNA 6000 Nano Bioanalyzer (Agilent Technologies, Inc.) and by Qubit Fluorometer (RNA HS Assay Kit - Invitrogen) according to the manufacturer's instructions. miRNA-Seq libraries were prepared using 4 μg of total RNA and Illumina's TruSeq small RNA Library Prep Kit. Single-end reads 1×75 bp, equal to 10.000.000 reads/sample, were sequenced on Illumina NOVASeq 6000.

Processing of miRNA-seq data

The nf-core/smrnaseq pipeline version 2.2.1 [19] was used to align sequencing data and build the miRNA count matrix used for differential expression analyses among in-study groups: pre-tx patients, post-tx patients with cancer and post-tx patients without cancer. The sequencing data were aligned using Bowtie1 to mature miRNA entries from the miRbase database and selecting the reference genome hg38.

Identification of differentially expressed miRNAs and miRNA expression profiles

Differential expression analyses were assessed using the functionalities of the R package *edgeR* [20–22] by fitting a negative binomial generalized log-linear model to the read counts for each miRNA. Then, miRNAs with an absolute log2 fold-change (FC) value greater than or equal to 1 and p−value less than 0.01 were selected as differentially expressed miRNAs (DEM) for each paired comparison. Three specific miRNA profiles were identified by intersecting the results of the differential analyses.

Enrichment analysis of validated miRNA targets

The validated target genes of the miRNAs identified for each profile were retrieved with the functionalities of *multiMiR* [23] (https://github.com/KechrisLab/multiMiR) and used to perform a gene set enrichment analysis with DAVID [24]. The biological processes (BPs) with a p-value less than 0.05 were selected for downstream analysis.

Expression of cancer-related miRNAs in the TCGA cohort

Expression data of cancer-related miRNAs (miR-1-3p, miR-10b-5p, miR-210-3p and miR-30a-3p) from seven studies of The Cancer Genome Atlas (TCGA) [https://www.cancer.gov/ccg/research/genome-sequencing/tcga] were retrieved using FireBrowse [https://gdac.broadinstiture.org/]. The studies were selected based on the distribution of cancer types among post-tx patients with cancer. The studies included data of tumor and normal solid tissue samples from patients with skin cancer melanoma (TCGA-SKCM), breast cancer (TCGA-BRCA), kidney renal clear cell carcinoma (TCGA-KIRC), kidney renal papillary cell carcinoma (TCGA-KIRP), thyroid carcinoma (TCGA-THCA) and prostate cancer (TCGA-PRAD). The studies for colorectal cancer (TCGA-COAD and TCGA-READ) were not selected because data on healthy tissues were unavailable. TCGA-SKCM was selected as the reference study for skin cancer, even if only two samples of healthy tissue were present in the cohort and most of the patients in our cohort had non-skin melanoma cancer. A student's t-test was performed to assess the differences in the expression of cancer-related miRNAs between the primary tumors and normal solid tissues.

Classification of the samples according to the miRNA expression profile

The miRNAs identified for each profile were used to set up two different classifiers: one based on the generalized linear (GLMnet) model and the other on the random forest model. The performance of the models was evaluated using the Leave-One-Out Cross-Validation (LOOCV) approach. *Caret* [25] was used for sample classification, while *ROCR* [26] was used to compute the Area Under the Curve (AUC). The optimal cut-off point for each classifier was estimated using the Youden Index (J) method [27].

Statistical analyses

All the statistical analyses were performed using R Statistical Software (v4.1.1, R Core Team 2021). Statistical analyses were conducted to evaluate the differential expression of circulating miRNAs across patient groups and to develop predictive models.

The likelihood ratio test implemented in the edgeR (v3.36.0) R package was applied to perform differential analysis. Serum miRNAs with an absolute log2 fold-change (FC) value greater than or equal to 1 and p−value less than 0.01 were selected as differentially expressed for all the analyses.

Fisher's Exact test adopted in the DAVID web server was used for gene set enrichment analysis. Biological processes with a p-value < 0.05 were selected for downstream analyses.

Student's t-test was used to compare the expression levels of cancer-related miRNAs in primary tumors and normal solid tissues of the TCGA datasets.

*R Core Team (2021) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.https://www.R-project.org.

Results

Characteristics of the cohort

We explored the serum miRNA profile of 138 patients, shown in the *circos* plot in Fig. 1, recruited from 4 Italian University Hospitals. Demographic and clinical data are summarized in Table 1. In particular, we collected the serum of 31 pre-tx patients, 30 post-tx patients with cancer, and 77 post-tx patients without evidence of cancer at the sampling time. The samples of the post-tx patients were collected at different time points after the kidney transplant. Among the post-tx patients with cancer, serum samples were obtained after cancer diagnosis and before the beginning of any cancer-specific treatment. Samples collected during remission or long after treatment were not considered. Therefore, the miRNA profiles observed likely represent long-term molecular alterations rather than acute tumor-associated changes. Most of the

post-tx patients with cancer were affected by skin cancer, mainly basal and squamous cell carcinoma.

Identification of differentially expressed circulating miRNAs

We examined the serum miRNA profiles of pre-tx patients and post-tx patients with and without cancer to investigate the variations associated with kidney transplant and to identify potential biomarkers for early cancer detection and risk assessment. We performed three pairwise comparisons: (1) post-tx with cancer versus pre-tx; (2) post-tx without cancer versus pre-tx; and (3) post-tx with cancer versus post-tx without cancer. We identified 67 DEMs (46 up-regulated and 21 down-regulated) in the first comparison, 146 DEMs (87 up-regulated and 59 down-regulated) in the second one, and 38 DEMs (4 up-regulated and 34 down-regulated) in the third one as shown in supplementary Tables 1 and 2 and in Fig. 2. In addition, we repeated the comparative analyses described above for samples collected 5 and 8 years after transplantation, considering these post-transplant time-points associated with a low kidney-transplant-related risk of
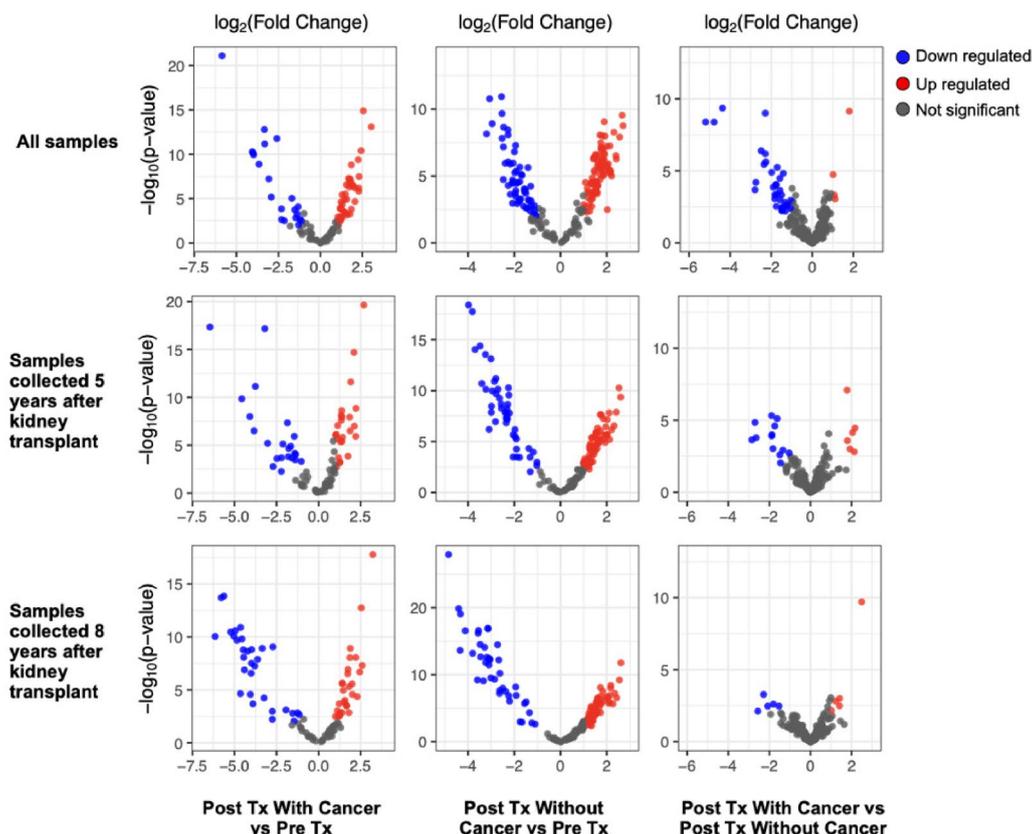

Fig. 2 Identification of differentially expressed serum miRNAs. The volcano plots show the differentially expressed serum miRNAs identified by comparing post-tx patients with cancer versus pre-tx patients (I column), post-tx patients without cancer versus the pre-tx patients (II column), and post-tx patients with cancer versus post-tx patients without cancer (III column). Each comparison was performed by selecting all post-tx patients (I row), post-tx patients sampled 5 years (II row) and 8 years (III row) after kidney transplant (II row). The red dots represent the up-regulated miRNAs, while the blue ones represent the down-regulated miRNAs. The miRNAs with |log2FC| >= 1 and p-value < 0.01 were selected as differentially expressed

cancer development according to available epidemiological reports [28] (Supplementary Tables 2 and 3, and Fig. 2).

Identification of miRNA expression profiles related to kidney transplant and cancer development

The up-regulated (Fig. 3A top panel) and down-regulated miRNAs (Fig. 3A bottom panel) identified through the previous nine pairwise differential analyses were intersected separately to determine common serum miRNA profiles across the samples. Based on these analyses, we identified three miRNA profiles (Fig. 1B) supposed to be transplant-related (TR-profile), cancer-related (CR-profile), and cancer-protective (CP-profile).

The TR-profile (Fig. 3B top panel) was composed of 21 up-regulated miRNAs (miR-24-3p, miR-93-5p, miR-16-5p, miR-223-3p, miR-425-5p, miR-25-3p, miR-451a, miR-20a-5p, miR-148a-3p, miR-3074-5p, miR-27a-3p, miR-221-3p, miR-22-3p, let-7d-5p, miR-29c-3p, miR-199a-3p, miR-199b-3p, miR-30e-5p, miR-191-5p, miR-29a-3p, miR-146a-5p, miR-146a-5p) and 3 down-regulated miRNAs (miR-3916, miR-6873-3p, miR-1207-5p). This signature was observed every time we compared post-tx to pre-tx patients. Based on this observation, we hypothesized that the changes in the expression levels of these circulating miRNAs could be a consequence of kidney transplant procedures and/or the immunosuppressive treatment.

The CR-profile (Fig. 3B middle panel) was composed of 1 up-regulated miRNA (miR-210-3p) and 3 down-regulated miRNAs (miR-30a-3p, miR-1-3p, miR-10b-5p). All the miRNAs of the CR-profile were identified when post-tx patients with cancer were compared to post-tx patients without cancer. The miR-10b-5p was also down-regulated when post-tx patients were compared to pre-tx patients.

The CP-profile (Fig. 3B bottom panel) was composed of 18 up-regulated miRNAs (miR-20b-5p, miR-454-3p, miR-194-5p, miR-32-5p, miR-192-5p, miR-17-5p, miR-424-5p, miR-532-5p, miR-182-5p, miR-363-3p, miR-26b-5p, miR-222-3p, miR-98-5p, miR-660-5p, miR-193a-5p, miR-92a-3p, miR-21-5p, miR-30a-5p) and 4 down-regulated miRNAs (miR-6746-3p, miR-765, miR-5193, miR-12116). This profile was identified when a group of post-tx patients without cancer was compared to the pre-tx patients; for this reason, we hypothesized that this signature could have a protective effect against cancer.

Identification of biological processes (BPs) regulated by the MiRNAs of each profile

We explored the BPs modulated by the up- and down-regulated miRNAs belonging to each profile to figure out their role in cancer development and kidney transplantation. To this aim, the validated target genes of the up- and down-regulated miRNAs of each profile were retrieved and used to perform a gene set enrichment analysis (Supplementary Table 3) with DAVID. We identified the BPs modulated by each set of target genes using a Fisher-Exact p-value < 0.05. Based on this criterion, we found that 911, 228 and 930 BPs were modulated by the target genes of up-regulated miRNAs of TR-profile, CR-profile and CP-profile, respectively. In contrast, 150, 585 and 128 BPs were regulated by the down-regulated miRNAs in the TR-profile, CR-profile and CP-profile, respectively. Among the top 20 BPs enriched by the target genes of up-regulated miRNAs belonging to CR-profile, we found the positive regulation of NLRP3 inflammasome, whose role in cancer initiation and development is emerging [29]. We also observed that the up-regulated miRNAs of each profile were involved in the regulation of signaling pathways associated with interleukins (ILs). 27 has been identified as a potential marker for the onset of post-transplant malignancies.

More specifically, the up-regulated miRNAs of the TR- and CR-profile regulated IL-6 production. Additionally, the up-regulated miRNAs of the CR- and CP-profile regulated IL-2 production, IL-6 signaling pathway and the cellular response to IL-4 and IL-1. The targets of up-regulated miRNAs in the TR-profile were also involved in the positive regulation of IL-10, IL-12, IL-8, IL-13, and IL-1β production. Furthermore, the targets of up-regulated miRNAs in the CP- and CR-profile were involved in the IL-15 and IL-27 signaling pathways, respectively. Interestingly, IL-27 has been identified as a potential marker for the onset of post-transplant malignancies [30].

In addition, we focused on the biological processes (BPs) involved in immune system functions, which relate to anti-rejection immunosuppressive therapy, and pigmentation-related pathways, linked to the high incidence of non-melanoma skin cancer in kidney transplant recipients (KTRs) In addition, we focused our attention on the BPs involved in immune system processes, which relate to anti-rejection therapy, and in pigmentation-related pathways, linked to the high incidence of non-melanoma skin cancer in kidney transplant recipients (KTRs) (Fig. 4). We found that the up-regulated miRNAs in the CP-profile were involved in T-helper 17 cell lineage commitment [31], while the down-regulated miRNAs were associated with B cell activation. Additionally, we observed that down-regulated miRNAs in the CR-profile and up-regulated miRNAs in the TR- and CP-profile played a role in the regulation of melanosome assembly. Furthermore, the up-regulated miRNAs in the CR-profile were involved in the regulation of melanosome transport and localization, whereas the up-regulated miRNAs in the CP-profile were associated with melanosome organization and endosome to melanosome transport.
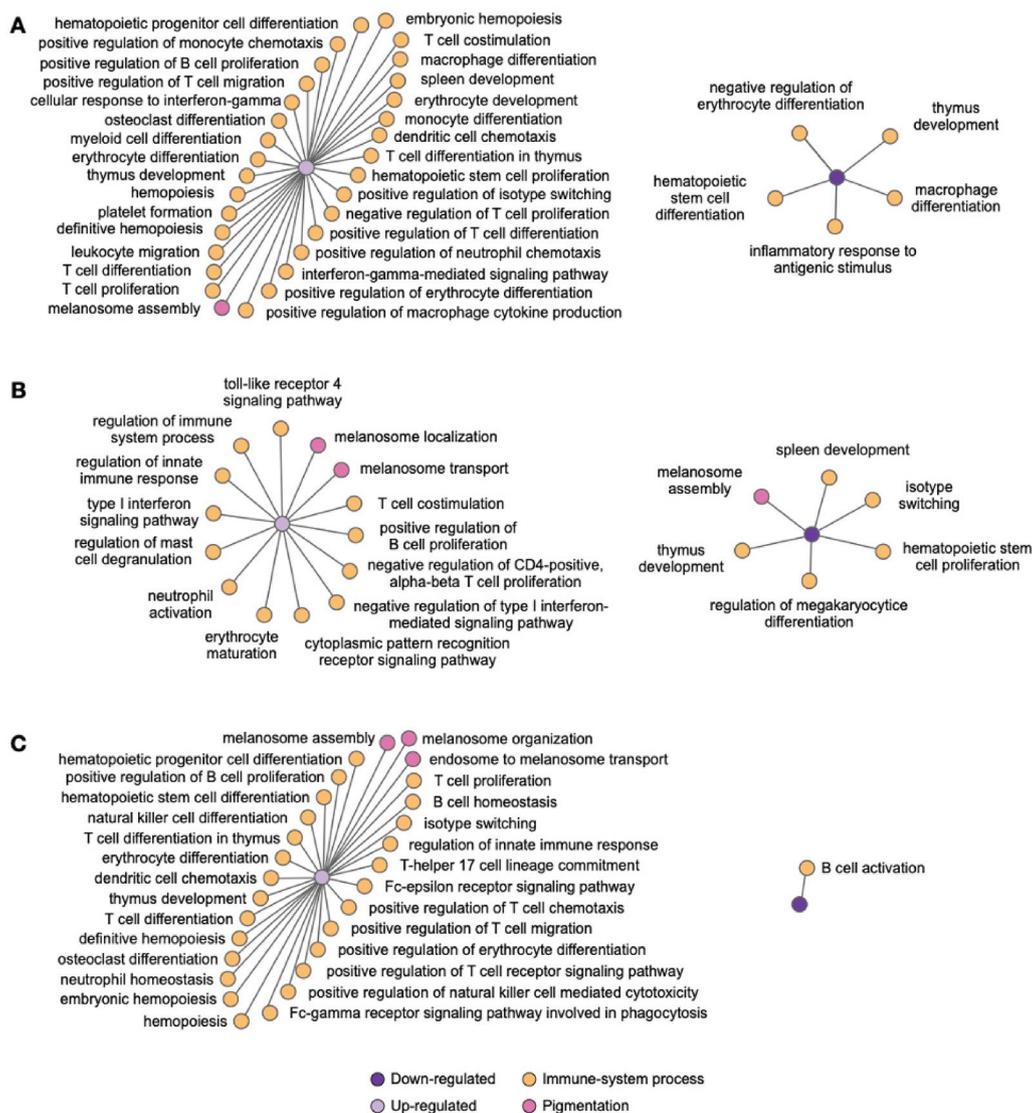
Fig. 3 Identification of 3 miRNA expression profiles. **A** The *UpSet* plots show the intersections of up-regulated miRNAs (top) and down-regulated miRNAs (bottom) identified for each pairwise comparison. The horizontal bar plot shows the number of DEM identified for each comparison, while the vertical one the number of common miRNAs among the analyses tagged with colored circles in the matrix. 'X' is equal to 'All' (all samples), '5' (samples collected 5 years after kidney transplant) or '8' (samples collected 8 years after kidney transplant) to indicate the samples included in the analysis. **B** Graphs of the TR-profile (top), CR-profile (middle) and CP-profile (bottom). The central node represents the profile type, while the neighbor nodes represent the up (light purple circles) and down-regulated (dark purple circles) miRNAs belonging to each profile

Validation of cancer-related miRNAs

We evaluated the expression levels of cancer-related miRNAs (miR-210-3p, miR-1-3p, miR-30a-3p and miR-10b-5p) across different tumor and healthy samples available in the TCGA Data Portal to validate their potential role in cancer diagnosis.

By analyzing the distribution of expression levels for each miRNA across tumor and healthy tissue samples in various TCGA cohorts (Fig. 5), we observed several notable patterns. MiR-210-3p was up-regulated in tumor tissue samples compared to the normal ones for all the cohorts except the TCGA-THCA ones, where it was

Fig. 4 Enrichment analysis. The graphs show the significant (p-value < 0.05) BPs belonging to the category of "immune-system process" (orange neighbor nodes) and "pigmentation" (pink neighbor nodes) enriched by the target genes of the up-regulated (light purple central node) and down-regulated (dark purple central node) miRNA of the **A** TR-profile, **B** CR-profile and **C** CP-profile

down-regulated. Conversely, in the TCGA-SKCM cohort no significant difference was found. MiR-1-3p consistently showed down-regulation in all tumor samples compared to healthy ones. MiR-30a-3p was generally down-regulated in tumor samples compared to healthy ones, except in the TCGA-PRAD and TCGA-SKCM cohorts, where no significant difference was observed. Lastly, MiR-10b-5p was down-regulated in all tumor samples, except for the TCGA-PRAD cohort, where it was up-regulated, and in the TCGA-SKCM cohort, where no significant difference was noted.

Development of binary classifiers for kidney transplant and cancer detection

The expression levels of miRNAs belonging to the TR-, CR-, and CP-profile were used to design multiple classifiers based on two different models: logistic regression (GLMnet) and random forest (rf). A first classifier was trained on the TR-miRNAs to evaluate whether this signature was able to classify pre-tx and post-tx samples, while a second and a third one were trained on the CR- and CP-miRNAs, respectively, to assess their capability to discriminate the samples of post-tx patients with cancer from those of post-tx patients without cancer. The performances of each binary classifier are illustrated in the ROC curves in Fig. 6, where the area under the ROC curve, the optimal cutoff (Youden Index), the specificity,
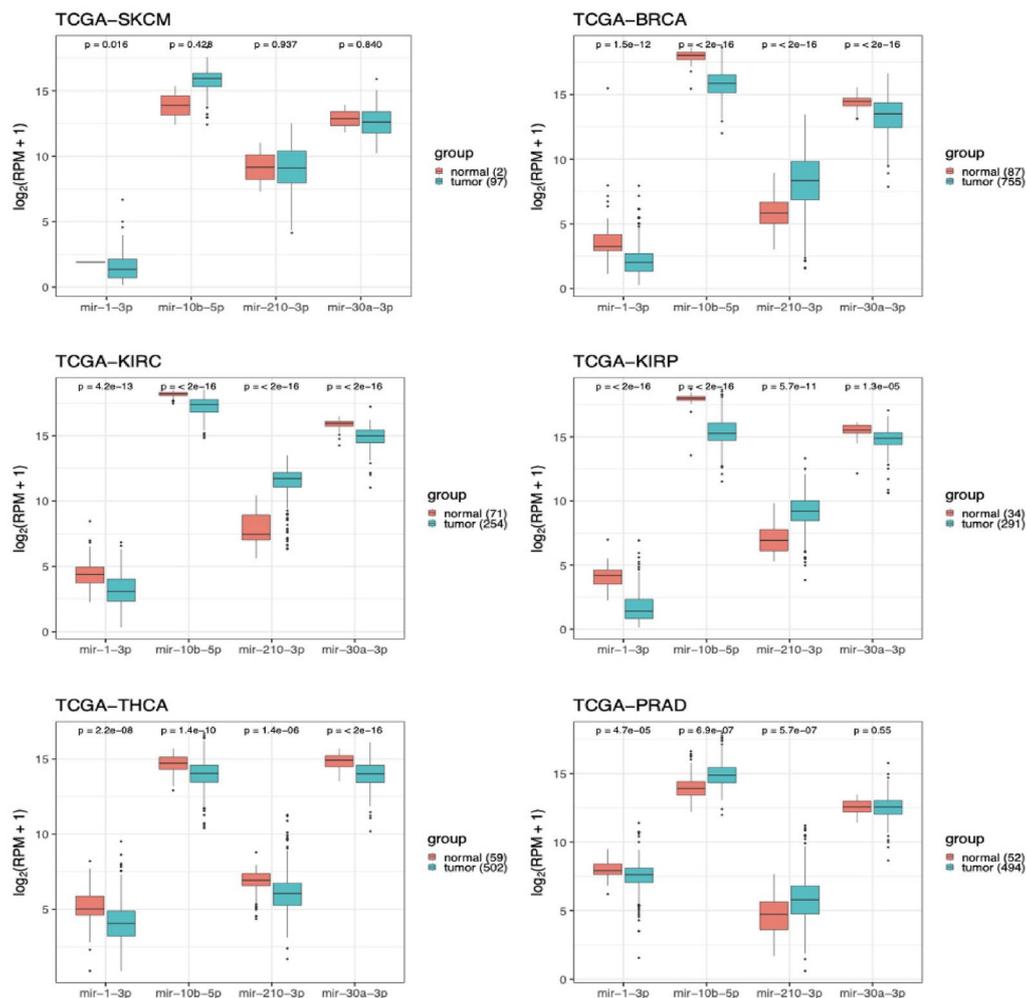
Fig. 5 Expression of cancer related miRNAs in TCGA cohort. The boxplots show the expression levels of the miR-1-3p, miR-10b-5p, miR-210-3p, miR-30a-3p across the normal and tumor tissue samples of the TCGA-SKCM, TCGA-BRCA, TCGA-KIRC, TCGA-KIRP, TCGA-THCA and TCGA-PRAD cohorts. The expression levels are represented as log2(RPM + 1)

and sensitivity are also reported for each classifier. We observed that the rf classifier based on the TR-miRNAs had a better performance compared to the one based on the GLMnet model (AUC: rf 0.877, AUC GLMnet: 0.807), while the classifier based on the GLMnet model and CR-miRNAs had a better performance compared to the rf classifier (AUC rf: 0.689, AUC GLMnet: 0.781). The classifiers based on the TP-miRNAs showed similar performances (AUC rf: 0.711, AUC GLMnet: 0.701).

Discussion

De novo cancers occurring in KTRs represent a potentially fatal complication, generally showing more aggressive behaviour than in the general population. Data from the Israel Penn International Transplant Tumours' Registry in the United States revealed that KTRs with colon cancer, non-small cell lung cancer, breast, prostate, bladder cancer, and kidney cancer had worse disease-specific survival compared to non-transplant patients with the

same cancer types [32]. Since the interaction between immunosuppressive protocols and anti-neoplastic drugs strongly influences the outcome of both transplant and cancer treatment, it represents a significant limiting factor in managing malignancies that occur in KTRs [33]. Early diagnosis has a pivotal role in oncology, especially in the post-transplant setting [34]. Developing specific, reliable immunological markers (soluble mediators, circulating miRNAs or specific circulating immune cell phenotypes) could significantly improve early diagnosis, potentially enhancing patients' survival while reducing morbidity and mortality. Additionally, understanding the immunological mechanisms undelying an increased neoplastic risk and the role of different immunosuppressive protocols may help clinicians in adjust immunosuppression strategies to prevent the development of post-transplant malignancies or to introduce specific treatments to preserve the anti-neoplastic immune response.
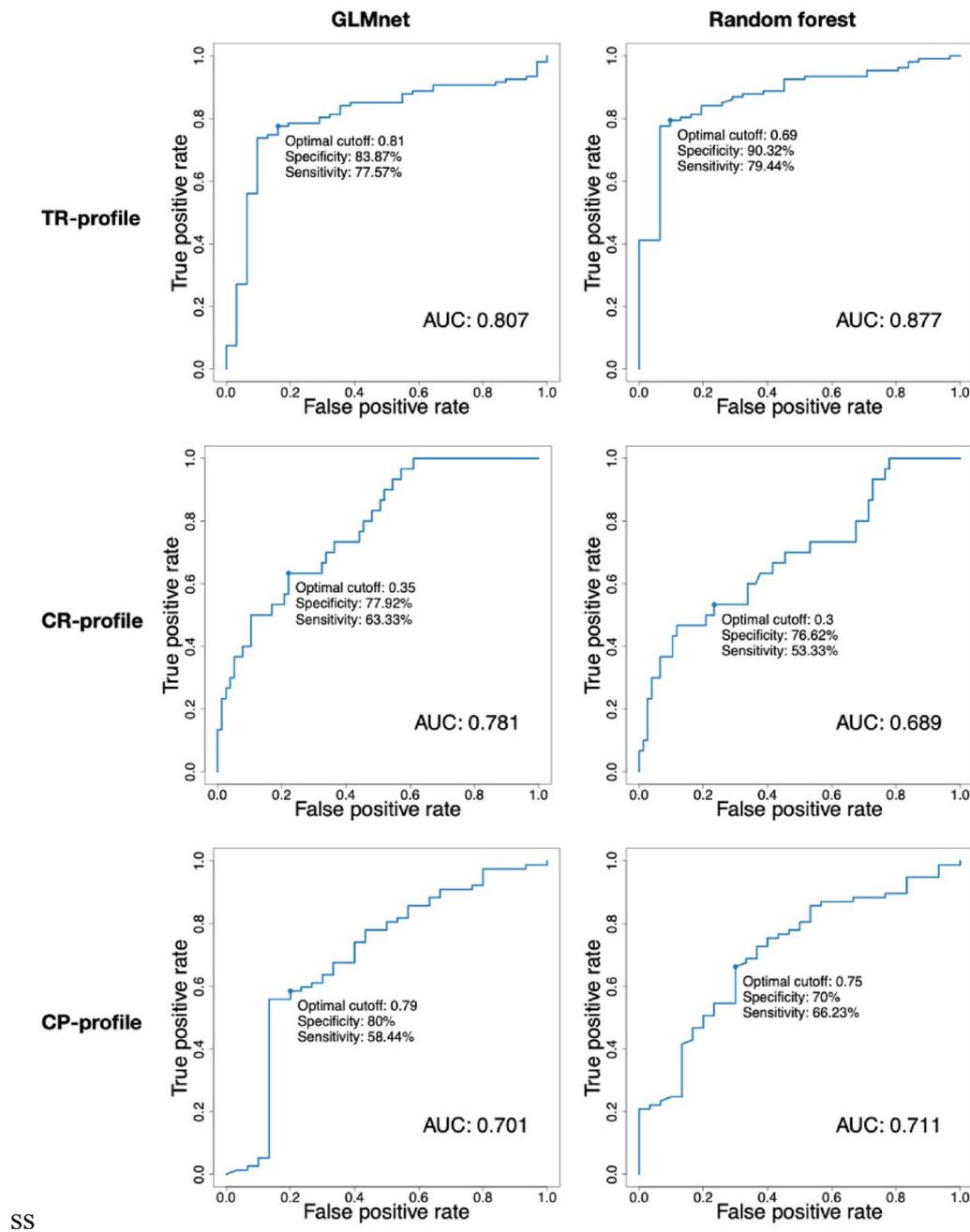
Fig. 6 Performance of classifiers built using the miRNA of TR-, CR- and CP-profile as features. The ROC curves show the performance of the classifiers based on the GLMnet model (left) and the random forest model (right) using the miRNAs of the TR-profile (I row), CR-profile (II row) and CP-profile (III row). The optimal cutoff (Youden Index), specificity and sensitivity are also reported for each classifier

COMETA study aimed to identify miRNA profiles associated with cancer in an Italian KTRs population from different national kidney transplant centres. We also aimed to validate our data and explore available literature on biological processes and molecular mechanisms associated with differentially expressed miRNAs identified in our analysis. The presence of a control group of KTRs that did not develop cancer, despite several years of exposure to transplantation, has introduced an important

chance for identifying miRNA profiles associated with protection or an increased risk for cancer in the presence of kidney transplant-related factors. Using an advanced high-throughput analysis, we were able to identify three relevant miRNA signatures in our population, despite the small sample size.

The TR-profile is particularly important, since it highlights the differential miRNA expression in relation to the exposure to the graft, its procedures and therapies

by comparing post-tx patients to pre-tx patients. This profile was characterized by the up-regulation or down-regulation of miRNAs relevant to the kidney transplant setting. Notably, miR-24-3p, miR-25-3p, miR-20a-5p and miR-27a-3p were up-regulated, and have been linked to oxidative stress-related ischemia-reperfusion injury [35–38]. Their upregulation may reflect the cold storage phase of kidney grafts before implantation, which could explain their increased levels NMSC in KTRs.

Additionally, the increased expression of miR-223-3p and miR-93-5p found in the TR-signature is in agreement with findings from other studies, supporting their relevance in transplant-related conditions [39].

MiR-146a-5p, also found up-regulated in the TR-profile, has been previously linked to graft rejection [40]. Similarly, up-regulated miR-29c-3p, miR-29a-3p, miR-30e-5p and miR-let-7d-5p have been associated to antibody-mediated graft rejection [41, 42], while miR-199a-3p and miR-199b-3p have been associated with T cell-mediated graft rejection [43]. The upregulation of miR-3074-5p, related to macrophage apoptosis, further supports immune response involvement [44]. MiR-148a-3p, miR-22-3p, and miR-191-5p are associated with acute kidney injury (AKI), and miR-16-5p might be an early biomarker for AKI or IgA nephropathy relapse in kidney transplants [45–51].

The CR-profile resulted from the comparison of post-tx patients with cancer with post-tx patients who did not develop cancer despite a long exposure to the kidney transplantation. The up-regulation of miR-210-3p was the distinctive finding of this signature, introducing a new insight on this interesting miRNA. To our knowledge, this is the first time that it has been found up-regulated in association with non-melanoma skin cancers (NMSC). Interestingly, many reports highlight its up-regulation in various other solid tumors, and its involvement in several pro-oncogenic pathways [52]. Recent studies showed that miR-210-3p is induced by hypoxia and HIF-1α, promoting epithelial-mesenchymal transition and increasing cell invasiveness by upregulating tumor growth factor (TGF)-β. This miRNA is also linked to chemoresistance, particularly to temozolomide in glioma cells. In lung adenocarcinoma, miR-210-3p serves as a potential non-invasive biomarker for diagnosis, and its silencing may reverse cancer progression. Additionally, miR-210-3p is elevated in prostate cancer bone metastasis and clear cell renal carcinoma, suggesting its role in tumor progression [53–56].

In 2021, Nashtahosseini et al. [57] reported significantly higher serum levels of miR-210-3p in breast cancer patients compared to healthy controls, although the exact mechanism remains unclear. A review by Maryam et al. linked miR-210 upregulation to increased VEGF, hypoxia, angiogenesis, and cell proliferation, migration,

and invasion in both Estrogen receptor (ER)-negative and ER-positive breast cancer, including triple-negative breast cancer [58]. Additionally, miR-210-3p expression was elevated in the serum of patient with pancreatic ductal adenocarcinoma (PDAC) compared to those with chronic pancreatitis and controls. Moreover, the three downregulated miRNAs in the CR-profile, miR-1-3p, miR-30a-3p and miR-10b-5p, are known to be tumor suppressors in several cancers [59–63]. MiR-1-3p exerts its tumor-suppressive function by different mechanisms in different neoplasms. Moreover, miR-30a-3p, also down-regulated in renal cell carcinoma tissues [64], PDAC [65], bladder cancer [66] and hepatocellular carcinoma [67], suppresses the tumor growth [68]. On the other hand, miR-10b-5p expression is reported to be lower in several uterine sarcomas and leiomyosarcomas compared to its expression in benign uterine tissues [69, 70]. However, unlike miR-1-3p and miR-30a-3p, which act as tumor suppressors and are downregulated in cancer, miR-10-5p seems to be up-regulated in other types of neoplasms, such as in glioblastoma [71] and in early stage hepatocellular carcinoma [72].

In the CP-profile, we compared miRNA expression in post-tx patients with cancer with post-tx patients without cancer. Among the up-regulated miRNAs in this signature, miR-424-5p has been well-studied in cancers like lung adenocarcinoma, breast, and hepatocellular carcinoma, where it regulates cell proliferation and the cell cycle, influencing tumor growth and survival [73, 74]. In liver transplantation, miR-424-5p has been linked to the recurrence of hepatocellular carcinoma [75]. On the other hand, miR-193a-5p is recognized as a tumor suppressor, with increased expression associated with reduced tumor proliferation and metastasis in several types of cancer [76]. Although its role in transplantation is not well-established, miR-193a-5p could be explored in the future as both a diagnostic marker and therapeutic target [77]. Our analysis also identified a downregulation of certain miRNAs in patients without cancer including, miR-12,116, miR-5193, miR-6476-3p, and miR-765 which were all downregulated. The most interesting data are related to miR-5193, which is involved in regulating of critical protective mechanisms for growth and immune evasion [78]. The increased incidence and aggressiveness of cancers in KTRs is not only a consequence of chronic immunosuppression but also the result of complex molecular and immunological alterations. Circulating miRNAs play a central role in modulating immune responses, controlling inflammation, and regulating both oncogenic and tumor-suppressor pathways. In the context of transplantation, miRNA dysregulation may reflect both immune activation against the graft and immunosuppression-induced escape of neoplastic clones [79]. Circulating microRNA profiles represent a promising

non invasive tool for improving the oncological management of KTRs. In the clinical setting, they could be used at several key points: for early diagnosis, thanks to their ability to detect tumor signals before clinical or radiological evidence; for risk stratification, identifying patients more susceptible to developing cancer in the pre- or early post-transplant phase and enabling personalized follow-up; and finally, for post-transplant monitoring, supporting longitudinal surveillance and the early detection of recurrence or new tumors. Integrating these biomarkers into clinical practice could, therefore, significantly improve timely diagnosis, personalized follow-up, and ultimately, patient outcomes. This study first supports the identification of three key miRNA signatures related to kidney transplant, including pro-oncogenic and onco-protective processes in a multicenter population for early diagnosis and personalized cancer treatments in KTRs. Moreover, there is currently no data available on the up-regulation of miR-210-3p in NSCM, which was a main finding of.

COMETA study.

Conclusion

Considering that the majority of patients with cancer in our study were affected by NMSC, and given its up-regulation found in all patients with cancer, we can firstly state that miR-210-3p is also up-regulated in patients with NMSC. Although our study is limited by its retrospective design and the inclusion of diverse cancer types within the studied population, the statistical significance of the identified miRNA signatures underscores their potential clinical importance. These findings provide a robust foundation for future prospective studies focused on KTRs, stratified by specific cancer types, as well as for rigorous validation studies to further confirm the utility of these miRNA signatures.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-025-07030-z.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Author contributions
M.A.S, M.Car., G.C., G.L.M., L.B., F.C.: conceptualization; M.A.S, R.T., V.G., A.M.C.: data curation; A.M.C, M.S., M.B.: Methodology and experiments; R.T.: bioinformatic analysis; M.A.S., R.T., A.M.C.: writing original draft preparation; A.C.: biobanca storage; G.C., M.A.S., A.M.C., C.A., A.M., M.Car: resources; M.A., V.G., C.A., R.P., G.P., P.M., G.C, A.M.: provided the samples, clinical information, and collected informed consent; M.A.S, M.Cecc, M.Car.: supervision; A.M.C.,

M.Cec., S.Z., M.Car., A.C., L.B., G.L.M., G.C., F.C.: review and editing. All authors have read and agreed to the published version of the manuscript.

Data availability
Datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study was conducted according to the Declaration of Helsinki and was approved by all local Ethics Committees of the participating centers (Approval nr 0029851/I – 2023 by the Ethical Committee of the Coordinating Centre at University of Campania "Luigi Vanvitelli").

Competing interests
All authors declare no conflicts of interest.

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References
1. Thongprayoon C, Hansrivijit P, Leeaphorn N, Acharya P, Torres-Ortiz A, Kaew-put W, et al. Recent advances and clinical outcomes of kidney transplantation. J Clin Med. 2020;9(4):1193.
2. D'Onofrio G, Simeoni M, Rizza P, Caroleo M, Capria M, Mazzitello G, Sacco T, et al. Quality of life, clinical outcome, personality and coping in chronic Hemodialysis patients. Ren Fail. 2017;39(1):45–53.
3. Wang Y, Hemmelder MH, Bos WJW, Snoep JD, de Vries APJ, Dekker FW, et al. Mapping health-related quality of life after kidney transplantation by group comparisons: a systematic review. Nephrol Dial Transpl. 2021;36(12):2327–39.

4.   Vajdic CM, van Leeuwen MT. Cancer incidence and risk factors after solid organ transplantation. Int J Cancer. 2009;125(8):1747–54.

5.   Piselli P, Serraino D, Segoloni GP, Sandrini S, Piredda GB, Scolari MP, et al. Risk of de Novo cancers after transplantation: results from a cohort of 7217 kidney transplant recipients, Italy 1997–2009. Eur J Cancer. 2013;49(2):336–44.

6.   Mohme M, Riethdorf S, Pantel K. Circulating and disseminated tumour cells - mechanisms of immune surveillance and escape. Nat Rev Clin Oncol. 2017;14(3):155–67.

7.   Gonzalez H, Hagerling C, Werb Z. Roles of the immune system in cancer: from tumor initiation to metastatic progression. Genes Dev. 2018;32(19–20):1267–84.

8.   Pellegrini B, Leone F, Greco R, Maggiolini M, Provenzano M, Zaza G. Endocrine malignancies: a still neglected issue in kidney transplantation. Front Med (Lausanne). 2025;17(12):1598168.

9.   Hao X, Lai W, Xia X, Xu J, Wu Y, Lv C, et al. Skin cancer outcomes and risk factors in renal transplant recipients: analysis of organ procurement and transplantation network data from 2000 to 2021. Front Oncol. 2022;12:1017498.

10. Postow MA, Callahan MK, Wolchok JD. Immune checkpoint Blockade in cancer therapy. J Clin Oncol. 2015;33(17):1974–82.

11. Wilson RAM, Evans TRJ, Fraser AR, Nibbs RJB. Immune checkpoint inhibitors: new strategies to checkmate cancer. Clin Exp Immunol. 2018;191(2):133–48.

12. Gong J, Chehrazi-Raffle A, Reddi S, Salgia R. Development of PD-1 and PD-L1 inhibitors as a form of cancer immunotherapy: a comprehensive review of registration trials and future considerations. J Immunother Cancer. 2018;6(1):8.

13. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med. 2015;373(17):1627–39.

14. Manohar S, Thongprayoon C, Cheungpasitporn W, Markovic SN, Herrmann SM. Systematic review of the safety of immune checkpoint inhibitors among kidney transplant patients. Kidney Int Rep. 2019;5(2):149–58.

15. Bocchetti M, Scrima M, Melisi F, Luce A, Sperlongano R, Caraglia M, et al. LncRNAs and immunity: coding the immune system with noncoding oligo-nucleotides. Int J Mol Sci. 2021;22(4):1741.

16. Cossu AM, Melisi F, Noviello TMR, Pasquale LS, Grisolia P, Reale C, et al. MiR-449a antagonizes EMT through IL-6-mediated trans-signaling in laryngeal squamous cancer. Mol Ther Nucleic Acids. 2024;35(1):102140.

17. Chakrabortty A, Patton DJ, Smith BF, Agarwal P. MiRNAs: potential as biomark-ers and therapeutic targets for cancer. Genes (Basel). 2023;14(7):1375.

18. Cortez MA, Anfossi S, Ramapriyan R, Menon H, Atalar SC, Aliru M, et al. Role of MiRNAs in immune responses and immunotherapy in cancer. Genes Chromosomes Cancer. 2019;58(4):244–53.

19. Ewels PA, Peltzer A, Fillinger S, Patel H, Alneberg J, Wilm A, et al. The nf-core framework for community-curated bioinformatics pipelines. Nat Biotechnol. 2020;38(3):276–8.

20. Chen Y, Lun AT, Smyth GK. From reads to genes to pathways: differential expression analysis of RNA-Seq experiments using Rsubread and the edger quasi-likelihood pipeline. F1000Res. 2016;5:1438.

21. McCarthy DJ, Chen Y, Smyth GK. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. Nucleic Acids Res. 2012;40(10):4288–97.

22. Robinson MD, McCarthy DJ, Smyth GK. EdgeR: a bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 2010;26(1):139–40.

23. Ru Y, Kechris KJ, Tabakoff B, Hoffman P, Radcliffe RA, Bowler R, et al. The MultiMiR R package and database: integration of microRNA-target interac-tions along with their disease and drug associations. Nucleic Acids Res. 2014;42(17):e133.

24. Sherman S, Hao BT, Qiu M, Jiao J, Baseler X, Lane MW. DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic Acids Res. 2022;50(W1):W216–21.

25. Kuhn M. Building predictive models in R using the caret package. J Stat Softw. 2008; 28, 1–26.

26. Sing T, Sander O, Beerenwinkel N, Lengauer T. ROCR: visualizing classifier performance in R. Bioinformatics. 2005;21(20):3940–1.

27. YOUDEN WJ. Index for rating diagnostic tests. Cancer. 1950;3(1):32–5.

28. Dhakal P, Giri S, Siwakoti K, Rayamajhi S, Bhatt VR. Renal cancer in recipients of kidney transplant. Rare Tumors. 2017;9(1):6550.

29. Zhiyu W, Wang N, Wang Q, Peng C, Zhang J, Liu P, et al. The inflammasome: an emerging therapeutic oncotarget for cancer prevention. Oncotarget. 2016;7(31):50766–80.

30. Pontrelli P, Rascio F, Zaza G, Accetturo M, Simone S, Infante B, et al. Interleu-kin-27 is a potential marker for the onset of post-transplant malignancies. Nephrol Dial Transpl. 2019;34(1):157–66.

31. McAllister F, Kolls JK. Th17 cytokines in non-melanoma skin cancer. Eur J Immunol. 2015;45(3):692–4.

32. Wong G, Chapman JR, Craig JC. Death from cancer: a sobering truth for patients with kidney transplants. Kidney Int. 2014;85(6):1262–4.

33. Coupier A, Gallien Y, Boillot O, Walter T, Guillaud O, Vallin M, et al. Antineo-plastic chemotherapy and immunosuppression in liver transplant recipients: squaring the circle? Clin Transpl. 2023;37(1):e14841.

34. Yildirim Y, Ozyilkan O, Emiroglu R, Demirhan B, Karakayali H, Haberal M. Early diagnosis of cancer in renal transplant patients: a single center experience. Asian Pac J Cancer Prev. 2006;7(2):336–9.

35. Tingle SJ, Sewpaul A, Bates L, Thompson ER, Shuttleworth V, Figueiredo R, et al. Dual MicroRNA Blockade increases expression of antioxidant protec-tive proteins: implications for ischemia-reperfusion injury. Transplantation. 2020;104(9):1853–61.

36. Zhang Y, Zuo X. miR-25-3p protects renal tubular epithelial cells from apoptosis induced by renal IRI by targeting DKK3. Open Life Sci. 2021;16(1):1393–404.

37. Shi L, Song Z, Li Y, Huang J, Zhao F, Luo Y, et al. MiR-20a-5p alleviates kidney ischemia/reperfusion injury by targeting ACSL4-dependent ferroptosis. Am J Transpl. 2023;23(1):11–25.

38. Zhao XR, Zhang Z, Gao M, Li L, Sun PY, Xu LN, et al. MicroRNA-27a-3p aggravates renal ischemia/reperfusion injury by promoting oxidative stress via targeting growth factor receptor-bound protein 2. Pharmacol Res. 2020;155:104718.

39. Ulbing M, Kirsch AH, Leber B, Lemesch S, Münzker J, Schweighofer N, et al. MicroRNAs 223-3p and 93-5p in patients with chronic kidney disease before and after renal transplantation. Bone. 2017;95:115–23.

40. Abbasi A, Ebadi P, Yaghobi R, Karimi MH. Association of MicroRNA146a G > C and MicroRNA196a-2 C > T gene polymorphisms with outcome of kidney transplantation in Iranian patients. Iran J Allergy Asthma Immunol. 2020;19(6):624–31.

41. Heinemann FM, Jindra PT, Bockmeyer CL, Zeuschner P, Wittig J, Höflich H et al. Glomerulocapillary miRNA response to HLA-class I antibody in vitro and in vivo. Sci Rep. 2018;8(1):6003. https://doi.org/10.1038/s41598-018-23760-1.]. Sci Rep. 2017;7(1):14554.

42. Franzin R, Stasi A, Sallustio F, Bruno S, Merlotti G, Quaglia M, et al. Extracel-lular vesicles derived from patients with antibody-mediated rejection induce tubular senescence and endothelial to mesenchymal transition in renal cells. Am J Transpl. 2022;22(9):2139–57.

43. Bijkerk R, Florijn BW, Khairoun M, Duijs JMGJ, Ocak G, de Vries APJ, et al. Acute rejection after kidney transplantation associates with Circulating MicroRNAs and vascular injury. Transpl Direct. 2017;3(7):e174.

44. Cong L, Zhao L, Shi Y, Bai Y, Guo Z. Circ_0006476 modulates macro-phage apoptosis through the miR-3074-5p/DLL4 axis: implications for Notch signalling pathway regulation in cardiovascular disease. Aging. 2024;16(16):11857–76.

45. Li X, Liu Y, Zhang Y, Hong Z. miR-425-5p inhibits rat mesangial cells (MCs) proliferation by targeting transforming growth factor beta (TGF-β)/RelA (p65) under the condition of sitagliptin. J Biomaterials Tissue Eng. 2020;10:545–53.

46. Kumari M, Mohan A, Ecelbarger CM, Gupta A, Prasad N, Tiwari S. miR-451 loaded exosomes are released by the renal cells in response to injury and associated with reduced kidney function in human. Front Physiol. 2020;11:234.

47. Saejong S, Townamchai S, Somparn N, Tangtanatakul P, Ondee P, Hirankarn T. MicroRNA-21 in plasma exosome, but not from whole plasma, as a biomarker for the severe interstitial fibrosis and tubular atrophy (IF/TA) in post-renal transplantation. Asian Pac J Allergy Immunol. 2022;40(1):94–102.

48. Ledeganck KJ, Gielis EM, Abramowicz D, Stenvinkel P, Shiels PG, Van Crae-nenbroeck AH. MicroRNAs in AKI and kidney transplantation. Clin J Am Soc Nephrol. 2019;14(3):454–68.

49. Sonoda H, Lee BR, Park KH, et al. MiRNA profiling of urinary exosomes to assess the progression of acute kidney injury. Sci Rep. 2019;9(1):4692.

50. Ge QM, Huang CM, Zhu XY, Bian F, Pan SM. Differentially expressed MiRNAs in sepsis-induced acute kidney injury target oxidative stress and mitochondrial dysfunction pathways. PLoS ONE. 2017;12(3):e0173292.

51. Wang X, Wang Y, Kong M, Yang J. MiR-22-3p suppresses sepsis-induced acute kidney injury by targeting PTEN. Biosci Rep. 2020;40(6):BSR20200527.

52. Liu H, Chen C, Zeng J, Zhao Z, Hu Q. MicroRNA-210-3p is transcriptionally upregulated by hypoxia induction and thus promoting EMT and chemoresistance in glioma cells. PLoS ONE. 2021;16(7):e0253522.

53. Ren D, Yang Q, Dai Y, Guo W, Du H, Song L, et al. Oncogenic miR-210-3p promotes prostate cancer cell EMT and bone metastasis via NF-κB signaling pathway. Mol Cancer. 2017;16(1):117.

54. Burnett RM, Craven KE, Krishnamurthy P, Goswami CP, Badve S, Crooks P, et al. Organ-specific adaptive signaling pathway activation in metastatic breast cancer cells. Oncotarget. 2015;6(14):12682–96.

55. Park BK, Zhang H, Zeng Q, Dai J, Keller ET, Giordano T et al. NF-kappaB in breast cancer cells promotes osteolytic bone metastasis by inducing osteoclastogenesis via GM-CSF. Nat Med 2007;13(1):62–9.

56. Petrozza V, Pastore AL, Palleschi G, Tito C, Porta N, Ricci S, et al. Secreted miR-210-3p as non-invasive biomarker in clear cell renal cell carcinoma. Oncotarget. 2017;8(41):69551–8.

57. Nashtahosseini Z, Aghamaali MR, Sadeghi F, Heydari N, Parsian H. Circulating status of MicroRNAs 660-5p and 210-3p in breast cancer patients. J Gene Med. 2021;23(4):e3320.

58. Maryam M, Naemi M, Hasani SS. A comprehensive review on oncogenic MiRNAs in breast cancer. J Genet. 2021;100:15.

59. Du G, Yu X, Chen Y, Cai W. MiR-1-3p suppresses colorectal cancer cell proliferation and metastasis by inhibiting YWHAZ-Mediated Epithelial-Mesenchymal transition. Front Oncol. 2021;11:634596.

60. Chen Y, Liu M, Jin H, Peng B, Dai L, Wang S, et al. Synthetic evaluation of MicroRNA-1-3p expression in head and neck squamous cell carcinoma based on microarray chips and MicroRNA sequencing. Biomed Res Int. 2021;2021:6529255.

61. Zhou S, Han H, Yang L, Lin H. MiR-1-3p targets CENPF to repress tumor-relevant functions of gastric cancer cells. BMC Gastroenterol. 2022;22(1):145.

62. Li T, Wang X, Jing L, Li Y. MiR-1-3p inhibits lung adenocarcinoma cell tumorigenesis via targeting protein regulator of cytokinesis 1. Front Oncol. 2019;9:120.

63. Li SM, Wu HL, Yu X, Tang K, Wang SG, Ye ZQ, Hu J. The putative tumour suppressor miR-1-3p modulates prostate cancer cell aggressiveness by repressing E2F5 and PFTK1. J Exp Clin Cancer Res. 2018;37(1):219.

64. Chen Y, Zhou J, Wu X, Huang J, Chen W, Liu D, et al. miR-30a-3p inhibits renal cancer cell invasion and metastasis through targeting ATG12. Transl Androl Urol. 2020;9(2):646–53.

65. Shimomura H, Okada R, Tanaka T, Hozaka Y, Wada M, Moriya S, et al. Role of miR-30a-3p regulation of oncogenic targets in pancreatic ductal adenocarcinoma pathogenesis. Int J Mol Sci. 2020;21(18):6459.

66. Hwang TI, Chen PC, Tsai TF, Lin JF, Chou KY, Ho CY, et al. Hsa-miR-30a-3p overcomes the acquired protective autophagy of bladder cancer in chemotherapy and suppresses tumor growth and muscle invasion [published correction appears in. Cell Death Dis. 2022;13(5):514.

67. Wang W, Lin H, Zhou L, Zhu Q, Gao S, Xie H, et al. MicroRNA-30a-3p inhibits tumor proliferation, invasiveness and metastasis and is downregulated in hepatocellular carcinoma. Eur J Surg Oncol. 2014;40(11):1586–94.

68. Tanigawa K, Misono S, Mizuno K, Asai S, Suetsugu T, Uchida A, et al. MicroRNA signature of small-cell lung cancer after treatment failure: impact on oncogenic targets by miR-30a-3p control. Mol Oncol. 2023;17(2):328–43.

69. Gonzalez Dos Anjos G, de Almeida L, Gomes de Almeida BC, Mourão T, Lavorato Rocha A, De Nardo Maffazioli G, et al. Could MiRNA signatures be useful for predicting uterine sarcoma and carcinosarcoma prognosis and treatment? Cancers (Basel). 2018;10(9):315.

70. Yoshida K, Yokoi A, Kitagawa M, Sugiyama M, Yamamoto T, Nakayama J, et al. Downregulation of miR–10b–5p facilitates the proliferation of uterine leiomyosarcoma cells: A MicroRNA sequencing–based approach. Oncol Rep. 2023;49(5):86.

71. Du D, Chen W, Wei D, Yu K, Gan D, Xu Z. MiR-10b-5p impairs TET2-Mediated Inhibition of PD-L1 transcription thus promoting immune evasion and tumor progression in glioblastoma. Tohoku J Exp Med. 2023;260(3):205–14.

72. Cho HJ, Eun JW, Baek GO, Seo CW, Ahn HR, Kim SS, et al. Serum Exosomal microrna, miR-10b-5p, as a potential diagnostic biomarker for early-stage hepatocellular carcinoma. J Clin Med. 2020;9(1):281.

73. Ghafouri-Fard S, Askari A, Hussen BM, Taheri M, Akbari Dilmaghani N. Role of miR-424 in the carcinogenesis. Clin Transl Oncol. 2024;26(1):16–38.

74. Wu J, Fu G, Luo C, Chen L, Liu Q. Cuproptosis-related CeRNA axis triggers cell proliferation and cell cycle through CBX2 in lung adenocarcinoma. BMC Pulm Med. 2024;24(1):85.

75. Wu L, Yang F, Lin B, Chen X, Yin S, Zhang F, et al. MicroRNA-424 expression predicts tumor recurrence in patients with hepatocellular carcinoma following liver transplantation. Oncol Lett. 2018;15(6):9126–32.

76. Wang JT, Wang ZH. Role of miR-193a-5p in the proliferation and apoptosis of hepatocellular carcinoma. Eur Rev Med Pharmacol Sci. 2018;22(21):7233–9.

77. Loosen SH, Wirtz TH, Roy S, Vucur M, Castoldi M, Schneider AT, et al. Circulating levels of microRNA193a-5p predict outcome in early stage hepatocellular carcinoma. PLoS ONE. 2020;15(9):e0239386.

78. Kamiya H, Komatsu S, Takashima Y, Ishida R, Arakawa H, Nishibeppu K, et al. Low blood level of tumour suppressor miR-5193 as a target of immunotherapy to PD-L1 in gastric cancer. Br J Cancer. 2024;130(4):671–81.

79. Liu X, Shen J, Yan H, Hu J, Liao G, Liu D, et al. Posttransplant complications: molecular mechanismsand therapeutic interventions. MedComm. 2024;5(9):e669.

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