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Diagnostic Value of Combination of MicroRNA-192 in Urinary Sediment and B-Ultrasound for Bladder Cancer

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Abstract

Objective: We aimed to explore the diagnostic value of microRNA-192 expression in urinary sediment combined with B-ultrasound in the diagnosis of bladder cancer. **Methods:** A total of 118 patients with bladder cancer and 120 patients with benign urinary system diseases were selected for collection of urinary sediment. Real-time quantitative polymerase chain reaction was applied to detect the microRNA-192 expression (normalized to U6 level) in urinary sediment. Besides, the relationship between microRNA-192 expression and clinicopathological characteristics was analyzed. Furthermore, receiver operating characteristic curve was performed to analyze clinical value of microRNA-192 expression alone and microRNA-192 expression in urinary sediment combined with B-ultrasound in the diagnosis of bladder cancer. **Results:** MicroRNA-192 expression was significantly downregulated in urinary sediment of patients with bladder cancer, which was related to tumor stage and tumor size ($P < .05$). The results of receiver operating characteristic curve analysis showed that the best critical value of microRNA-192 expression in urinary sediment for the diagnosis of bladder cancer was 0.785 with the sensitivity and specificity of 76.7% and 78.0%, respectively. The sensitivity and specificity of microRNA-192 expression in urinary sediment combined with B-ultrasound in the diagnosis of bladder cancer were 93.2% and 76.7%, respectively. The sensitivity of combined diagnosis (93.2%) was not significantly different from that of cystoscopy (93.2%; $P > 0.05$). There were significant differences between the expression of microRNA-192 in urinary sediment and the sensitivity of B-ultrasound examination alone with cystoscopy ($P < .05$). **Conclusion:** The downregulation of microRNA-192 expression in urinary sediment of patients with bladder cancer may be related to tumor progression. The microRNA-192 expression in urinary sediment is valuable in the diagnosis of bladder cancer, which shows high sensitivity in diagnosis of bladder cancer when combined with B-ultrasound.

Keywords

microRNA-192, bladder cancer, B-ultrasound, urinary sediment, diagnostic value, combined diagnosis

Abbreviations

AUC, atypical urothelial cells; CA, carcinoma; CLL, chronic lymphocytic leukemia; miR, microRNA; NEG, negative; qPCR, quantitative polymerase chain reaction; ROC, receiver operating characteristic; SUCA, suspicious carcinoma

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Introduction

Bladder cancer is known as one of the commonest cancers of the genitourinary organs in the world and the seventh leading cause of cancer-related deaths.[1] It is estimated that bladder cancer is the 10th most frequently diagnosed cancer in both males and females and the mortality rates keep stable in the last decade.[2] Bladder cancer has many known risk factors, including cigarette smoking, chemicals (aniline dyes), and other exposures, although many cases arise with no apparent

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exposure to carcinogens.[3] About 70% to 80% of newly diagnosed patients with bladder cancer develop nonmuscle invasive diseases, and after endoscopic and intravesical treatment, 50% to 70% of patients recur and 10% to 30% of people develop invasive muscle disease.[4] Bladder cancer is usually distinguished by blood visible in urine or blood found in urine tests, but emergency care is a common approach for bladder cancer and is often correlated with poor prognosis.[5] As the gold standard for bladder cancer follow-up, cystoscopy should be conducted every 3 months for the first 2 years, every 6 months for the next 2 years, and then every year according to clinical judgment and patient's preference.[5] Additionally, the development of ultrasound makes the diagnosis of bladder cancer more accurate and can be included in the follow-up plan of patients with superficial bladder cancer.[6]

Evidence reports that microRNAs (miRs) are dysregulated in bladder cancer and may act as noninvasive urine markers for primary diagnosis of bladder cancer.[7] Previous evidence also demonstrated that miRs and proteins that reflect the properties of bladder cancer could be found in urine samples of patients with bladder cancer.[8] Aberrant expression of miR-192 has been observed in various kinds of cancers. Especially, overexpression of miR-192 could induce apoptosis of bladder cancer cells, suggesting its tumor suppressive role in bladder cancer.[9] Interestingly, the patients with bladder cancer have a lower expression of miR-192 in their urinary sediment.[10] Besides, conventional B-ultrasound is the recommended imaging method for lymph node diseases with the advantages of high-resolution and real-time evaluation.[11] B-mode ultrasound as the basis of examination is wildly applied for screening papillary thyroid carcinoma (CA).[12] It is reported that B-ultrasound and cystoscopy are 2 conventional diagnostic tools for bladder cancer, which are need to supplement or supplant.[13] There is no report focused on miR combined with B-ultrasound for the diagnosis of bladder cancer. Thus, in our study, we applied quantitative polymerase chain reaction (qPCR) to detect differential expression of miR-192 in urinary sediment of patients with bladder cancer and healthy controls. Besides, we combined the expression of miR-192 in urinary sediment and B-ultrasound examination in the diagnosis of early bladder cancer for the first time to compare the sensitivity and specificity of single and combined examination, which provided a new theoretical basis for the selection of diagnostic mode of early bladder cancer.

Materials and Methods

Study Patients

A total of 118 patients with bladder cancer were selected with 15 mL morning urine collected from each patient. All patients were pathologically diagnosed with bladder cancer.[14] None of the patients with bladder cancer received any adjuvant treatment nor had systemic diseases or other organs of benign or malignant tumors. At the same time, the morning urine samples of 120 patients with benign urinary system diseases were collected as the control group. Within 30 minutes after collecting urine samples, the samples were centrifuged at a speed of 2000*g* for 10 minutes with the urine sediment separated. After that, RNA was extracted via mirVanaTm PARIS miRNA extraction kit (ABI Company, Foster City, California) after washing with 500 μL phosphate-buffered saline for 2 times. The RNA extraction was stored at −80°C for further use.

B-Mode Ultrasound

Patients had bladder filling status with supine position, and the tumors in bladder were scanned through the abdominal wall above the symphysis pubis and at various angles with linear array probe (frequency of 3.5 MHz). Besides, the upper abdomen and the back of the waist at all angles were also scanned to carefully observe the tumors in the kidney and ureter. After examination, cystoscopy was performed. If suspicious lesions were found, biopsy was performed, followed by pathological examination (including cystoscopy and routine pathological examination of postoperative specimens).

Urinary Cytology

The slides were fixed in a special centrifugal tube and urine samples were put into the centrifugal tube and centrifuged at 1700 rpm in CF-120 centrifugal machine for 5 minutes. The slides were taken out and stained with Papanicolaou staining. After obtaining the cytological smears, the slides were dehydrated in anhydrous ethanol for 10 minutes, cleared in xylene for 1 minute, and then sealed and sent to the pathologist for microscopic examination. All specimens were randomly reviewed by experienced cytopathologists and the results were reported. Urinary cytological diagnosis can be divided into 4 categories: (1) negative (NEG): the epithelial cell changes were in the normal range, no atypical cells and malignant cells; (2) atypical urothelial cells (AUC): the atypia of epithelial cells was more obvious than that of reactivity, but there was no definite malignant feature; (3) suspicious carcinoma (SUCA): changes in epithelial cells were malignant but not sufficient in quantity or quality to diagnose cancer; (4) CA: epithelial cells with malignant features. In statistical analysis, NEG and AUC in cytological results were classified as negative diagnosis, and SUCA and CAs were classified as positive diagnosis.

Real-Time Quantitative Polymerase Chain Reaction

The above extracted RNA was reverse transcribed based on the instructions of Taqman miRNA reverse transcription kit (ABI Company). Real-time qPCR was applied for analysis with the primer sequences of miR-192: forward: 5′-GCGGCG-GCTGACCTATGAATTG-3′; and reverse: 5′-ATCCAGTGC AGGGTCCGAGG-3′; U6: forward: 5′-TCCGATCGT -GA AGCGTTC-3′; and reverse: 5′-GTGCAGGGTCCGAGGT-3′. The reaction conditions were at 50°C for 2 minutes, at 95°C for 10 minutes, at 95°C for 10 seconds, at 60°C for 60 seconds with a total of 45 cycles. ABI PRISM 7900 system (ABI Company) was used for automatic analysis. The relative expression of

Table 1. Comparisons of Baseline Characteristics Between the Case and Control Groups.[a]

Baseline Characteristics	Control (n = 120)	Case (n = 118)	*P*
Age (years)	56 (40-69)	58 (43-72)	.192
Gender			.546
Male	79	82	
Female	41	36	
Tumor stage			–
Ta	–	65	
T1	–	33	
T2	–	20	
Grade			–
Low	–	45	
High	–	73	
Tumor size (cm)			–
≤3	–	68	
>3	–	50	

[a]Mann-Whitney *U* test and χ^2 test.

miR-192 was detected via $2^{-\triangle\triangle Ct}$ method, with U6 as the internal reference.

Statistical Analysis

All statistical analyses were performed using SPSS version 19.0 software (IBM Corp, Armonk, New York). The measurement data consistent with the normal distribution were expressed as mean ± standard deviation, and pairwise comparison between 2 groups was analyzed by independent *t* test. Others were expressed as median (min, max). Mann-Whitney *U* and Wilcoxon rank sum test were applied to compare gene expression levels between groups. The counting data were expressed as percentage and analyzed by χ^2 test. The receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value of miR-192 level (<0.785 as positive), B-ultrasound (in positive), or miR-192 combined with B-ultrasound (miR-192 level <0.785 and B-ultrasound was positive) in bladder cancer, and the Youden index (sensitivity + specificity − 1) was used to determine the best critical value for detection of bladder cancer. Value of $P < .05$ was considered to be of statistical significance.

Results

Comparisons of Baseline Characteristics Between the Case and Control Groups

In the case group, the median age of patients was 58 years, ranging from 43 to 72 years, including 82 males and 36 females. There was no significant difference in age, sex, and benign urinary system diseases between the case group and the control group ($P > .05$). Among 118 patients with bladder cancer, there were 65 cases at Ta stage, 33 cases at T1 stage, and 20 cases at T2 stage; 45 cases at low grade and 73 cases at high grade; 68 cases had tumor size ≤3 cm and 50 cases had tumor size >3 cm (Table 1).

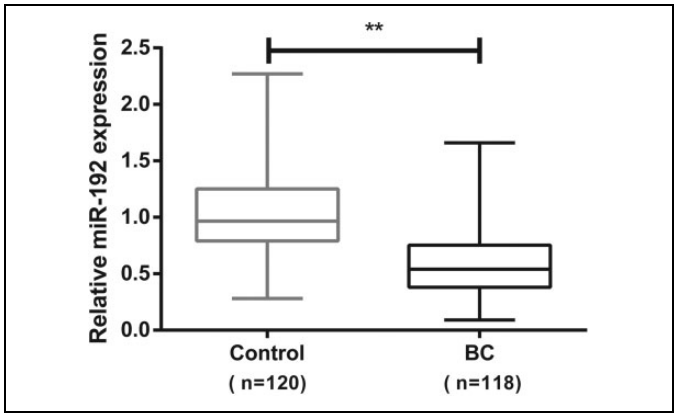

Figure 1. Detection of microRNA-192 expression in urinary sediment of patients in the case and control groups by quantitative polymerase chain reaction. Note: Mann-Whitney *U* test; $^{**}P < .001$.

Downregulation of miR-192 Expression in Urinary Sediment of Patients With Bladder Cancer

Quantitative polymerase chain reaction was used to detect the expression of miR-192 in urinary sediment of the case and control groups. The results showed that the relative expression of miR-192 in urinary sediment of the case group was (0.54 [0.09-1.66]) lower than that of the control group (0.965 [0.28-2.27]; Figure 1). These results suggest that the expression of miR-192 in urinary sediment of patients with bladder cancer is downregulated.

Expression of miR-192 in Urinary Sediment of Patients With Bladder Cancer Is Related to Tumor Progression

The relationship between the expression of miR-192 in urinary sediment and clinicopathological features in patients with bladder cancer was further analyzed. The results showed that the expression of miR-192 in urinary sediment was related to tumor stage and tumor size. The higher the tumor stage, the lower the expression of miR-192 in urinary sediment. The expression of miR-192 in urine sediment of patients with tumor size >3 cm was significantly lower than that of patients with tumor size ≤3 cm ($P < .001$). The results of urinary cytology showed that the lower the level of miR-192, the higher the positive rate of urinary cytology for bladder cancer (all $P < .001$). However, the expression of miR-192 in urinary sediment of patients with bladder cancer was not related to age, sex, and malignant grade ($P > .05$; Table 2). It is suggested that the expression of miR-192 in urinary sediment of patients with bladder cancer is related to the progression of tumor.

Expression of miR-192 in Urinary Sediment Combined With B-Ultrasound Has Certain Diagnostic Value for Bladder Cancer

The optimal critical value of miR-192 expression in urinary sediment for the diagnosis of bladder cancer was 0.785 with

Table 2. Relationship Between miR-192 Expression and Clinicopathological Characteristics in Urinary Sediment of Patients With Bladder Cancer.[a]

	Cases	miR-192	*P*
Age (years)			.272
≤55	50	0.555 (0.13-1.66)	
>55	68	0.525 (0.09-1.56)	
Gender			.321
Male	82	0.54 (0.09-1.66)	
Female	36	0.55 (0.16-1.56)	
Tumor stage			<.001
Ta	65	0.65 (0.39-1.66)	
T1	33	0.525 (0.16-1.38)	
T2	20	0.295 (0.09-0.38)	
Grade			.654
Low	45	0.56 (0.22-1.66)	
High	73	0.53 (0.09-1.57)	
Tumor size (cm)			<.001
≤3	66	1.93 (0.29-1.66)	
>3	52	0.52 (0.09-1.49)	
Urinary cytology			<.001
NEG	35	0.63 (0.36-1.66)	
CA	83	0.48 (0.09-1.38)	

Abbreviations: CA, carcinoma cell; miR-192, microRNA-192; NEG, negative; the epithelial cell changes were in the normal range, no atypical cells and malignant cells.
[a]Mann-Whitney *U* test and Wilcoxon rank sum test to compare gene expression levels between groups.

the sensitivity and specificity of 76.7% and 78.0%, respectively, and AUC value was 0.796 (Figure 2A). The sensitivity and specificity of B-ultrasound alone in the diagnosis of bladder cancer were 73.7% and 95.8%, respectively, and AUC value was 0.848 (Figure 2B). The sensitivity and specificity of miR-192 expression in urinary sediment combined with B-ultrasound in the diagnosis of bladder cancer were 93.2% and 76.7%, respectively, and AUC value was 0.954 (Figure 2C). The sensitivity of combined diagnosis (93.2%) was not significantly different from that of cystoscopy ($P > .05$). Using 0.785 as the best critical value of miR-192 expression in urinary sediment for the diagnosis of bladder cancer, the results of miR-192, B-ultrasound, combination (miR-192 + B-ultrasound), cystoscopy, and urinary cytology test are shown in Table 3. Further analysis showed that there was no significant difference between the sensitivity of miR-192 expression in urine sediment and the single examination of B-ultrasound with cystoscopy ($P > .05$; Table 4). The results demonstrated that the expression of miR-192 in urinary sediment combined with examination of B-ultrasound showed high sensitive to the diagnosis for bladder cancer.

Discussion

Bladder cancer requires expensive invasive methods for diagnosis and follow-up, leaving as a burden for health system.[15] Previous evidence revealed that changes in miRs can be detected noninvasively and quantified in body fluids and miRs are good molecular markers for diagnosis, prognosis, and clinical follow-up.[16] Therefore, the discovery and utilization of urine-based noninvasive markers for the diagnosis of bladder cancer attracted our attention. In our study, we explored the diagnostic value of miR-192 expression in urinary sediment combined with B-ultrasound in the diagnosis of bladder cancer. Finally, we demonstrated that the miR-192 expression in urinary sediment is valuable in the diagnosis of bladder cancer, which shows high sensitivity in the diagnosis of bladder cancer when combined with B-ultrasound.

Our study enrolled 118 patients with bladder cancer with their morning urine collected to detect the expression of miR-192 in urinary sediment. The result showed that downregulated miR-192 expression was found in urinary sediment of patients with bladder cancer. MicroRNA-192 is downregulated in lung cancer tissues, and upregulated miR-192 expression could suppress lung cancer cell proliferation.[17] In line with our study, Jin *et al* also proved that overexpression of miR-192 significantly

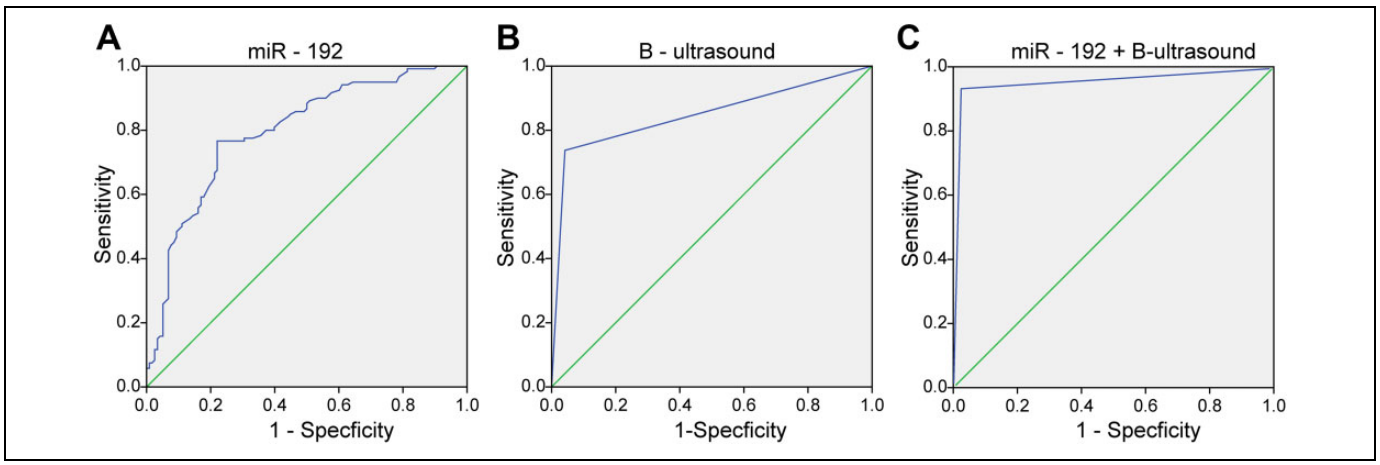

Figure 2. The receiver operating characteristic (ROC) curve for diagnostic value of microRNA-192, B-ultrasound, and combination of miR-192 and B-ultrasound for bladder cancer. A, The ROC curve for diagnostic value of miR-192. B, The ROC curve for diagnostic value of B-ultrasound. C, The ROC curve for diagnostic value of combination of miR-192 and B-ultrasound.

Table 3. Diagnostic Value of miR-192, B-Ultrasound, Combination of miR-192 and B-Ultrasound, and Cystoscope for Patients in the Case and Control Groups.

	miR-192		B-Ultrasound		Combination (miR-192 + B-Ultrasound)		Cystoscope		Urinary Cytology	
	≤0.785	>0.785	+	−	+	−	+	−	+	−
Control (n = 120)	28	92	5	115	3	117	4	116	0	120
Case (n = 118)	92	26	87	31	110	8	110	8	83	35

Abbreviation: miR-192, microRNA-192.

Table 4. Comparison of Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value of Each Method in the Diagnosis of Bladder Cancer.

	miR-192	B-Ultrasound	Combination (miR-192 + B-Ultrasound)	Cystoscope	Urinary Cytology
Sensitivity	78.0	73.7	93.2	93.2	70.3
Specificity	76.7	95.8	97.5	96.7	100.0
Positive predictive value	76.7	94.5	97.3	96.5	100.0
Negative predictive value	78.0	78.8	93.6	93.4	77.4

Abbreviation: miR-192, microRNA-192.

inhibited the proliferation of bladder cancer cells.[9] Subsequently, the relationship between the expression of miR-192 in urinary sediment and clinicopathological features in patients with bladder cancer was further analyzed, with the results presented that the expression of miR-192 in urinary sediment was related to tumor stage and tumor size. We provided evidence that expression of miR-192 in urinary sediment of patients with bladder cancer was related to tumor progression. Accumulating evidences have been demonstrated that many miRs could play a crucial role in tumorigenesis, progression, and metastasis of cancer cells.[16-18] Interestingly, urine appears to be a good source for detection of miR due to its content of cell-free nucleic acid in supernatants or precipitates.[19] Previous report proved that miR-192-overexpressing cells exhibited a significant increase in G0/G1 phase and a significant decrease in S phase in bladder cancer.[9] One study focused on miR-192 and colorectal cancer reported that miR-192 expression significantly decreased with increasing colorectal cancer stage and being lowest in stage IV tumors.[20]

In the following, we evaluated the ROC curve of miR-192 expression in urinary sediment and B-ultrasound, respectively, for the diagnosis of bladder cancer. The results showed that the optimal critical value of miR-192 expression in urinary sediment for the diagnosis of bladder cancer was 0.785 with the sensitivity and specificity of 76.7% and 78.0%, respectively, while the sensitivity and specificity of B-ultrasound alone in the diagnosis of bladder cancer were 73.7% and 95.8%, respectively. After that, we combined the miR-192 expression in urinary sediment of patients with bladder cancer with B-ultrasound, and consequently, we found that the sensitivity and specificity of miR-192 expression in urinary sediment combined with B-ultrasound in the diagnosis of bladder cancer were 93.2% and 76.7%, respectively. Diagnostic role of miR-192 was also proved in human chronic lymphocytic leukemia (CLL), with reduced expression ($\sim$2.5-folds) found in patients with CLL.[21] Ultrasound is a simple and rapid examination that has nothing to do with any complications inherent in cystoscopy and allows safe examination of all individuals without any restrictions; besides, it is also easily available, cost-effective, and a noninvasive technique without requiring special preparation, which can provide images of both the upper and lower renal tract.[6] It is reported that B-ultrasound and cystoscopy are 2 conventional diagnostic tools for bladder cancer.[13] By examining and evaluating the cytological sections of urinary sediments stained with Papanicolaou, the researchers in their previous study proved the relationship between urinary tract hyperplasia and infectious hematoma.[22] Based on ultrasound examination, known biomarkers of adult suffered from bladder cancer and infected with chronic schistosomiasis are associated with bladder damage.[23] Our study also clarified that the sensitivity of combined diagnosis (93.2%) was not significantly different from that of cystoscopy ($P > .05$). Thus, we speculated that the combination of expression of miR-192 in urinary sediment combined with examination of B-ultrasound showed high sensitive to the diagnosis of bladder cancer. Consistently, the sensitivity and specificity of combined application of ultrasound, urinary CYFRA21-1, and cytology were 90.5% and 67.2% respectively, thus serving as an effective and noninvasive method for detecting recurrent bladder tumors.[24]

There are several standard methods for bladder cancer screening, and each has its advantages and disadvantages. For example, urinary cytology is minimally invasive but not so precise (sensitivity: 40%-60% and specificity: 90%-100%), but the sensitivity of this test significantly increases with malignancy grade.[25] However, the main concern considering metabolomics in urine as a diagnostic system is the variability of glomerular filtration, both with medication and dietary habits as the main confounding factors.[26] The biomarkers present in blood-based liquid biopsy hold great promise, as they are able to record and monitor the disease stage at real time and predict

prognosis, recurrence, therapy response, and resistance, without invasive intervention,[26] but the stability of miR in body fluid could affect the sample quality and miR expression.[25] Szarvas *et al* noted that in screening bladder cancer, urine sediment alone had a sensitivity of 68%, while urine supernatant alone indicated aberrations in 80% of the tumors, indicating the analysis of free DNA in the urine supernatant provides a higher detection rate.[27] According to these references, we needed to do more researches to evaluate the best miR-based test for bladder cancer screening.

In conclusion, downregulated miR-192 expression was found in urinary sediment of patients with bladder cancer after qPCR detection, which is related to the progression of tumor. Furthermore, we made a conclusion that expression of miR-192 in urinary sediment combined with examination of B-ultrasound showed high sensitive to the diagnosis for bladder cancer. However, further follow-up was needed to evaluate the role of combination of miR-192 and B-ultrasound in the prognosis of bladder cancer.

Authors' Note

This study was approved by the Institutional Research Ethics Committee of the Faculty of Medicine, China-Japan Union Hospital, Jilin University (approval no. 2016-wjw009). All patients provided written informed consent prior to enrollment in the study.

Declaration of Conflicting Interests

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