Clinicopathological and prognostic involvements of MCT1, MCT4 and IL-7R in esophageal squamous cell carcinoma

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Abstract

Background: MCTs, short for monocarboxylate transporters, especially MCT1 and MCT4, have been widely touched upon in a variety of immune and cancer cells; however, they have been rarely described in esophageal squamous cell carcinoma (ESCC). IL7R, receptor of interleukin 7, has been shown to operate in several cancers; though, the clinicopathological implication of IL7R expression in ESCC remains less known.

Methods: Herein, to understand the clinicopathological involvements of MCT1, MCT4 and IL7R in ESCC, immunohistochemistry was performed with ESCC tissue microarray comprising 86 paired ESCC and its matched normal control dots. Post-hoc statistical analyses were undertaken with Cross-table and survival analyses. Results: It was shown that concomitant expression of MCT1, MCT4 and IL7R prevailed in the stromal compartment of ESCC tissues relative to the epithelial. Moreover, up-regulated MCT1 and MCT4 were markedly associated with tumor size; while IL7R was displayed to closely correlate with lymph node metastases and clinical stage. Elevated MCT1, MCT4 and IL7R were strikingly associated with adverse outcome of ESCC.

Conclusions: Together, the data we presented here indicate that MCT1/4 and IL7R were heavily involved in the oncogenesis of ESCC.

Background

Monocarboxylic acid, including lactate, pyruvate and ketone bodies, have been found to play major roles in cancer metabolism and must be transported across both the plasma and mitochondrial membranes [1], where monocarboxylate transporter (abbreviated as MCT) family actually take charge of the process. MCT family now comprises as many as 14 members, of which only the first four, that is, from MCT1 to MCT4, have been experimentally borne out to catalyze the transport of monocarboxylates, such as lactate, pyruvate and ketone bodies [1, 2], which come to be recognized as important metabolic fuel for cancer cells. Given this, there is a need to understand the clinicopathological as well as prognostic implication of MCT1 and MCT4 expression in ESCC, where it has been little described surrounding MCT1 and MCT4 expression and its significance.

Lactate, as mentioned above, has been shown to be as an important metabolic fuel for normal cells in certain physiological setting [3] and in cancer [4–6] as well. Recently, it has begun to be recognized as active molecule capable of modulating the immune response [7–10]. The interleukin-7 receptor (IL7R), commonly expressed in immune cells, has been suggested to be critical in survival, development and homeostasis in the immune system [11]. Advanced genome-wide cancer studies have reported that IL7R is genetically amplified in human esophageal squamous cell carcinoma (ESCC)[11]; yet, the clinicopathological involvement of IL7R in ESCC remains unknown.

Herein, to learn about the clinicopathological significance of MCT1, MCT4 and IL7R expression in ESCC, Immunohistochemistry was undertaken to concomitantly appraise the expression patterns of these three biomarkers of our interest with ESCC tissue microarray, followed by statistical analyses with clinicopathological variables comprising demographic, clinical stage, TNM classification and overall prognosis. It was shown that these three biomarkers we picked up predominated in ESCC tissues as compared with its paired normal controls and that over-expressed MCT1, MCT4 and IL7R expression were closely related to adverse outcome and metastases of ESCC.

Methods

Human specimens

This study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University. Written informed consent was obtained from each participant involved. The preparation of ESCC tissue microarrays used

for the immunostaining analysis of MCT1, MCT4 and IL7R were outsourced to Shanghai Outdo Biotech. Co. Ltd (Superchip, Shanghai, China). The tissue microarray was composed of 90 paired ESCC tissues and its matched normal dots. Notably, 4 paired cases of dots were missing in the experimental process. As a consequence, the real case number was 86 for each. Staging and grading of the sample tissues was graded according to hematoxylin and eosin (H&E) staining. Histopathological diagnosis was conducted following the criteria of the World Health Organization 2017 version. None of the samples were collected from patients undergoing chemoradiotherapy before esophagectomy. The clinicopathological variables comprising demographic, clinical stage, TNM classifications, and overall survival, were documented when available.

Immunohistochemistry (IHC)

In brief, the ESCC tissue microarrays were deparaffinized and rehydrated. Heat-induced epitope retrieval was performed using citrate buffer (pH=6.0) and a microwave histoprocessor (Haier, Qingdao, Shandong, China), after which the tissue sections were incubated with 3% hydrogen peroxide for 10 minutes to block endogenous peroxidase activity. Tissue sections were then incubated with a primary antibody to MCT1 (Catalog number: 20139-1-AP; dilution at 1:600, Proteintech, China), MCT4 (Catalog number: 22787-1-AP; dilution at 1:400, Proteintech, China) and IL7R (Catalog number: TA327014; dilution at 1:150, Origene, Rockville, MD, USA) overnight in a humidified chamber at 4°C. Immunostaining was visualized using a labeled horseradish peroxidase (HRP)-conjugated AffiniPure goat anti-rabbit IgG antibody (Catalog number: ZB-2301; ready-to-use format; ZSGB-Bio, Beijing, China) with 3, 3’-diaminobenzidine as a chromogen, and the tissues were counterstained with hematoxylin. Primary antibodies replaced with normal rabbit anti-human IgG antibody were used as negative control. Colorectal carcinoma tissues, as has been earlier reported [12], were served as positive control for MCT1 and MCT4 expression, and lung cancer tissue[13] was picked up as positive control for IL7R expression.

Immunoscoring

The immunoscoring of the three biomarkers we picked out was performed under a light microscope by two pathologists (WLC and ZY, appreciated in acknowledgement). The staining patterns were scored based on the intensity and area of the positive staining, as described elsewhere [14]. Specifically, the intensity of positive staining was scored as follows: 0, negative; 1, weak staining; 2, moderate staining; and 3, strong staining. The rate of positive cells was scored on a 0 to 4 scale: 0, 0–5%; 1, 6–25%; 2, 26–50%; 3, 51–75%; and 4, >75%. If the positive staining was homogeneous, a final score was achieved by multiplication of the two scores above, give birth to a total range from 0 to 12. When the staining was heterogeneous, it was scored as follows: each component scored independently and summed for the results. For example, a specimen containing 25% tumor cells with moderate intensity (1 × 2 = 2), 25% tumor cells with weak intensity (1× 1 = 1), and 50% tumor cells without staining received a final score of 2 + 1 + 0 = 3. Dispute, if any, arises in the scoring will be approached by the consensus reached between the two pathologists. For statistical analysis, all the samples of ESCC were categorized into two major groups according to positive expression as follows: scores of 0 to 8 as low expression and scores of 9 to 12 as high expression.

Statistical analysis

Chi-square was used in clinicopathological characteristics between MCT1, MCT4 and IL7R high and low expression. Notably, when expected number was less than 5, Fisher’s exact test was applied. For survival, the start date was the beginning of treatment, either esophagectomy or chemotherapy or radiotherapy, ending with the last follow-up date or

death. Overall survival was calculated using Kaplan-Meier curves, whereas survival differences between groups were examined by log rank tests. Multivariate analysis with Cox proportional-hazards modeling was performed to adjust for covariates. When P-value of <0.05 was considered statistically significant. Statistical analysis was conducted using SPSS 17.0 version (SPSS, Chicago, USA).

Results

MCT1, MCT4 and IL7R predominated in the stroma of ESCC.

To understand the expression of MCT1, MCT4 and IL7R in ESCC, IHC was conducted on ESCC tissue microarray consisting of 90 paired ESCC and its matched normal control dots. Considering the underlying problems regarding correctness and specificity that may be caused by the quality of primary antibody itself that were commercially available, we pre-examined the quality of the three primary antibodies to MCT1, MCT4 and IL7R following the approach, which is to say antigen pre-adsorption, mentioned by Hewitt SM and colleagues [15]. Pre-test result confirmed the specificity of the three primary antibodies commercially available were adequate to do their jobs (data not shown), which was corroborated by the negative control (Figure 1) and positive control (Figure 2) we purposefully set up from the outset. Subsequently, we set out to simultaneously detect the concomitant expression of MCT1, MCT4 and IL7R with IHC. Results from IHC revealed that the positive staining of MCT1, MCT4 and IL7R were mainly membranous and slightly cytoplasmic. The distribution of MCT1, MCT4 and IL7R were mainly confined to the stroma, as opposed to epithelial where little positive staining can be detectable. Intensity of immunostaining of MCT1, MCT4 and IL7R were highly heterogeneous, varying greatly from case to case, with intensity being from negative, weak, moderate and strong in ESCC tissues (Figure 3). Besides, the immunostaining of MCT1, MCT4 and IL7R can also be present in paired normal control tissues, with the intensity of the bulk of cases being weak staining. Only few cases were moderate. Despite present in both ESCC and its matched normal controls, the staining of MCT1, MCT4 and IL7R, taken as a whole, were appreciably over-expressed in ESCC relative to normal control (Table 1), explicitly indicating the tumor-promoting roles in ESCC.

Clinicopathological involvements of MCT1, MCT4 and IL7R in ESCC.

Having seen the immunohistochemical characteristics of MCT1, MCT4 and IL7R in ESCC tissues, next we sought to analyze the clinicopathological meaning of MCT1, MCT4 and IL7R expression by Cross-Table statistical analysis. It was exhibited that compared with the paired normal control, MCT1, MCT4 and IL7R were drastically over-expressed in ESCC tissues (Table 1). As stated already, the distribution of ESCC cells with positive immunostaining of MCT1, MCT4 and IL7R were predominantly confined to the stromal compartment. Only a few cases with positive immunostaining of these three markers can be visible in epithelial. Furthermore, there was significant correlation between lymph nodes metastases versus MCT1 (P=0.040) and IL7R (P=0.011) stromal expression. Among MCT1, MCT4 and IL7R, only MCT1 was shown to be pronouncedly associated with T classification (P=0.005), tumor size (P=0.047) and clinical stage (P=0.029). What's slightly different from MCT1 in that, MCT4 was displayed to be markedly associated with tumor size (P=0.044), yet no significant association can be identified with other clinicopathological variables. Notably, although no significant association can be reached between MCT4 stromal expression and age; there was a statistical trend towards significance (P=0.061). Regarding IL7R, stromal expression of IL7R was observed to markedly correlate with clinical stage (P=0.002) in addition to lymph nodes metastases (P=0.011). Still, no significant association can be achieved between stromal expression of IL7R and other clinicopathological variables, including distant metastases, differentiation, T classification, tumor size and demographic information consisting of age and gender.

Prognostic implications of MCT1, MCT4 and IL7R in ESCC.

Next, to understand the prognostic significance of MCT1, MCT4 and IL7R expression in ESCC, Kaplan-Meier survival curve was plotted to analyze the statistical difference between patients with high versus low expression of the three biomarkers we took interested in. Kaplan-Meier survival analysis exhibited that, there was statistically significant difference between patients with high expression and low expression of MCT1, MCT4 and IL7R (Figure 2), strong indicating that up-regulated MCT1, MCT4 and IL7R expression was significantly associated with unfavorable overall prognosis in patients with ESCC. To assess the effect of MCT1, MCT4 and IL7R expression and clinicopathological variables available on prognosis, both univariate and multivariate survival analyses were undertaken. Univariate Cox regression analysis revealed that MCT1 (P=0.001), MCT4 (P=0.001) and IL7R (P=0.001) expression, T classification (P=0.009), Clinical stage (P=0.025), and N classification (P=0.048) were prognostic factors in ESCC. By using multivariate analysis, we further stringently evaluated the prognosis related factors that were shown to be significant in univariate analysis. It exhibited that MCT1 (P=0.004), MCT4 (P=0.005) and IL7R (P=0.008) expression and T classification (p=0.013), lymph node metastasis (p=0.016), differentiation degree (P=0.036) were independent prognostic factors affecting the 5-year overall survival. Notably, despite no significant association can be obtained between clinical stage and outcome, there was a statistical trend towards significance (P=0.069). Together, the data explicitly indicate that MCT1, MCT4 and IL7R expressions can be used as a prognostic predictor in ESCC (Table 2).

Discussion

In this study, MCT1, MCT4 and IL7R expression were shown to be strikingly predominant in the stroma of ESCC tissues; and that over-expressed MCT1, MCT4 and IL7R were found to be significantly linked with T classification, lymph node metastasis and distant metastasis. Moreover, these three biomarkers were analyzed to be independent prognostic factors in ESCC, strongly suggestive of their prognostic value in ESCC.

As numerous studies have probed the biochemical or mechanistic roles of MCT1, MCT4 in the setting of cancer from different tissue origins, our aim here was not to explore their working mechanism but to analyze the clinicopathological implications of their expression in ESCC. Immunohistochemical characteristics were quantitatively analyzed of ESCC cells expressing MCT1, MCT4 and IL7R, exhibiting that immunostaining of MCT1, MCT4 and IL7R were observed mainly on cytoplasmic membrane and slightly in cytoplasm. In order to analyze the correlation between these three markers we take interested in, four sets of ESCC tissue microarrays were used on which dots of each set were entirely obtained from the same site of tissue serially sectioned. To rule out the possibility that the three biomarkers could be expressed by other types of cells, other than ESCC cells we focused upon only, within the tumor microenvironment; Hematoxylin and eosin (H&E) staining was performed and frequently reviewed when immunoscoring. Given this, the expression of MCT1, MCT4 and IL7R was thus unlikely to be overestimated in ESCC tissues in our setting.

MCT1, also known as SLC16A1, has been reported to take charge of influx of lactate. In stark contrast, physiologically, MCT4 was found to be in charge of efflux of lactate, as systemically reviewed by Halestrap AP[16]. Although extensive studies can be readily available regarding the MCT1 in cancers of different types; it has been little described in esophageal cancer with the exception of two recent related reports[17, 18]: one about MCT1 expression in Barrett's esophagus and adenocarcinoma, the other ESCC. Analysis from previous investigations concerning MCT1 in cancers, regardless of different types[19–22], revealed that MCT1 prevailed or predominantly over-expressed in cancerous tissues as compared with normal controls and up-regulated MCT1 was found to be linked with poor outcome as well as tumor metastases, which was fully in agreement with our observations in ESCC of our own case. What's congruent with our description with regard to MCT1 is that, in a recent study [18] by Chen X and colleagues, MCT1 was exhibited to be an independent prognostic factor in ESCC. What's different from the study by Chen X et al [18] in that, there was short of

functional analysis data of MCT1 in our setting with cell culture system. Examination of data from these aforementioned literatures along with our own findings about MCT1 in ESCC, explicitly suggested the prognostic value of MCT1 in malignancies, irrespective of their types.

Much like MCT1, MCT4 comes from the same family as MCT1 does. But, in the case of mediating the lactate transport, it was totally opposite of MCT1. MCT4 was mainly involved in the release of lactate or “efflux”, whereas uptake of lactate or “influx” was chiefly mediated by MCT1, as extensively reviewed [1, 23]. Relative to the quantity of studies of MCT1, MCT4 seemed to be received more attention than those of MCT1 in cancer. Studies undertaken in the area of cancer abound, unraveling that MCT4 was uniformly seen to be strikingly up-regulated in cancerous tissues compared with normal control; and elevated MCT4 was correlated with unfavorable outcome [19, 21, 24–26]. By contrast, there were only two pieces of literature with regard to MCT4 in esophageal cancer until now[17, 27]. In our findings, MCT4 was exhibited to be an independent prognostic factor of ESCC, whereas no significant correlation with lymph nodes metastases was observed, was partly supported by the replicated observation made by Cheng B et al in ESCC[27]. Moreover, we also noted that MCT4 expression was found to be closely related with tumor size, as MCT1 was. It may be that more lactate transporters could be needed so as to suit the metabolic reprogram with the development and progression of ESCC cells.

Scant data has been available on the clinicopathological implications of IL7R in solid tumors, not to mention in ESCC; although several lines of evidence existed concerning genetic polymorphisms or mutations of IL7R in the context of hematopoietic lymphoma [28]. IL7R was not necessarily expressed in immune cells; it can also be expressed in cancer cells, such ESCC cells [11] and hepatoma cells [29]. Although there was one similar study has been replicated regarding IL7R in ESCC [11]; the clinicopathological involvement of IL7R currently remains unknown. In our study, IL7R was shown to be over-expressed in ESCC tissues relative to paired normal controls, which was highly consistent with what has been reported by Kim MJ and associates [11]. Unfortunately, there has been a shortage of clinicopathological analysis of IL7R expression in their study. Hinted from the limited data available in the above studies, these findings suggest that IL7R can operate oncogenically in malignant tumors. Our observation that up-regulated IL7R was closely linked with clinical stage and shorter overall prognosis, further supports the oncogenic function of IL7R in tumors.

Current opinion holds that Inflammation is associated with the accumulation of lactate at sites of tumor-growth [30]. Several tumors and inflammatory sites have displayed accumulation of lactate and altered expression of its transporters, which is strongly suggestive of the heavy involvement of lactate transporters in cancer and inflammation [30]. In our study, despite we failed to analyze the lactate concentration on the ESCC tissue microarray that we used to concomitantly assess the expression of MCT1, MCT4 and IL7R owing to the technical limitation; over-expressed MCT4 can mean the release of lactate would be much more active than it should be. As a result, there will not be surprising that more lactate released from ESCC cells could stimulate inflammatory microenvironment in a way, thereby contributing to the up-regulation of IL7R of ESCC cells. More recently, a piece of evidence showing that lactate production was dramatically enhanced after colorectal cancer cells were treated with IL6 [31], strengthens the conjecture we raised here. Based on the observation, it can be safely reasoned that elevated IL7R may also boost the lactate yield in ESCC cells, which consequently may require more lactate transporters, such as MCT1 and MCT4, in order to suit any variation caused by lactate in tumor microenvironment. Of course, the hypothesis we posed here needs to be tested experimentally in the following. On the other, in consideration of lymphocyte that infiltrates the ESCC tissues can also express these three markers, it is therefore somewhat difficult to control the real expression of MCT1/4 and IL7R only in ESCC cells. As for the lymphocyte that infiltrates the ESCC tissues that may also express MCT1/4 and IL7R, which beyond the scope of our investigation.

Conclusion

In conclusion, our study presented that expression of MCT1, MCT4 and IL7R was closely linked with the adverse outcome of ESCC, suggesting that lactate transporters MCT1/4 and IL7R were heavily involved in the oncogenesis of ESCC.

Declarations

Acknowledgements

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Abbreviations:

MCTs, monocarboxylate transporters

ESCC, esophageal squamous cell carcinoma

IL7R, interleukin 7 receptor

IHC, Immunohistochemistry

HRP, horseradish peroxidase

SPSS, Statistical Product and Service Solutions

H&E, Hematoxylin and eosin.

Authors' contributions

STZ performed all the experiment and drafted the manuscript, QL and TL did some favours in immunohistochemistry, DDT and TXS aided in providing the reagents involved, XZ and XJH helped analyzing the statistical analysis, XML was in charge of the whole study.

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Availability of data and materials

The datasets used and/or analyzed during this study are available from the

corresponding author on reasonable request.

Ethics approval and consent to participate

This study was performed after being approved by the Medical Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University.

Consent for publication

Not applicable.

Competing interests

It is declared that all authors have no conflict of interest.

Footnotes

Publisher's Note

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Tables

Variables		N	MCT1 expression		χ^2	*P*	MCT4 expression		χ^2	*P*	IL7R expression		χ^2	*P*
			High	Low			High	Low			High	Low		
ANT		86	26	60	17.067	0.000	35	51	10.291	0.002	30	56	38.222	0.000
ESCC		86	53	33			56	30			70	16		
Gender	Male	60	48	12	1.179	0.282	39	21	0.001	1.000	50	10	0.492	0.550
	Female	26	18	8			17	9			20	6		
Age(years)	≥60	55	34	21	0.002	1.000	40	15	3.891	0.061	47	8	1.660	0.251
	≤60	31	19	12			16	15			23	8		
Clinical stage	I-II	61	33	28	5.031	0.029	37	24	1.838	0.218	55	6	10.654	0.002
	III	25	20	5			19	6			15	10		
T classification	T_1-T_2	49	24	25	7.705	0.005	30	19	0.759	0.494	39	10	0.245	0.781
	T_3-T_4	37	29	8			26	11			31	6		
N classification	N_0	34	16	18	5.047	0.040	19	15	2.111	0.170	23	11	7.019	0.011
	N_1-N_3	52	37	15			37	15			47	5		
Differentiation	Well	13	7	6	0.793	0.673	8	5	4.607	0.100	8	5	4.107	0.128
	Moderate	36	24	12			28	8			30	6		
	Poor	37	22	15			20	17			32	5		
Tumor size (cm^3)	≥1	12	5	7	6.136	0.047	6	6	6.229	0.044	9	3	0.627	0.731
	1—3	50	38	12			38	12			42	8		
	≥3	24	20	14			12	12			19	5		
Gross classification	Ulcerative	40	26	14	0.406	0.816	27	13	1.891	0.388	35	5	2.496	0.287
	Fungating	9	5	4			4	5			6	3		
	Medullary	37	22	15			25	12			29	8		

Table 1. The clinicopathological involvements of MCT1, MCT4 and IL7R in ESCC.

Note: ANT, adjacent normal tissue; Fisher's exact test was used in cells where expected value was less than 5 in the contingency table.

Table 2. Univariate and multivariate Cox regression analysis of variables related with the prognosis in ESCC.

Variables	Univariate		Multivariate	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Gender				
male *vs* female	1.212 (0.486-2.225)	0.474		
Age				
≤60 *vs* >60	0.867 (0.505-1.486)	0.602		
Tumor size (cm^3)				
≤3 *vs* >3	0.659 (0.341-1.356)	0.274		
T classification				
T_1-T_2 *vs* T_3-T_4	0.375 (0.184-0.755)	0.009	0.411 (0.165-0.947)	0.036
Differentiation				
Well/moderate-poor	0.634 (0.371-1.362)	0.239		
Clinical stage				
I-II *vs* III	0.517 (0.314-0.926)	0.025	0.600 (0.345-1.041)	0.069
N classification				
N_0 *vs* N_1-N_3	2.023(0.947-4.180)	0.048		
MCT1 expression				
High *vs* Low	0.334 (0.169-0.523)	0.001	0.338 (0.212-0.727)	0.004
MCT4 expression				
High *vs* Low	0.271 (0.142-0.519)	0.001	0.410 (0.222-0.767)	0.005
IL7R expression				
High *vs* Low	0.216 (0.124-0.557)	0.001	0.364 (0.157-0.827)	0.008

Note: *vs*, versus; HR, hazard ratio; CI, confidence interval.

Figures

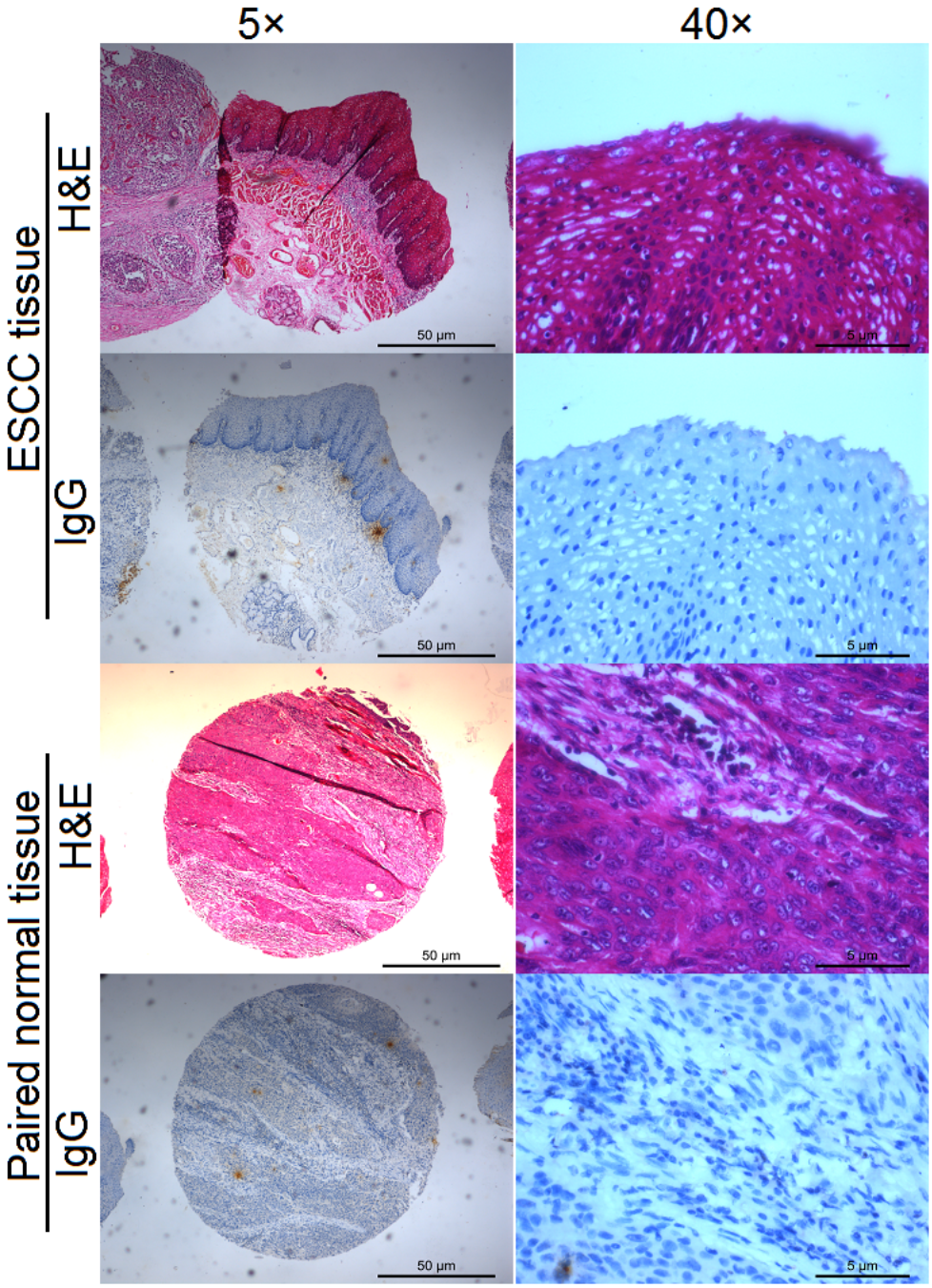

Figure 1

The negative control set up in the immunohistochemistry in ESCC. The primary antibody was replaced with normal rabbit IgG (Catalog number: ZM-0491; clonality: 4E3; ZSGB-Bio, Beijing, China), diluted at 1:150, was as negative control. The original magnification was 50 fold; magnification of the field of interest was 400 fold. H&E, hematoxylin-eosin.

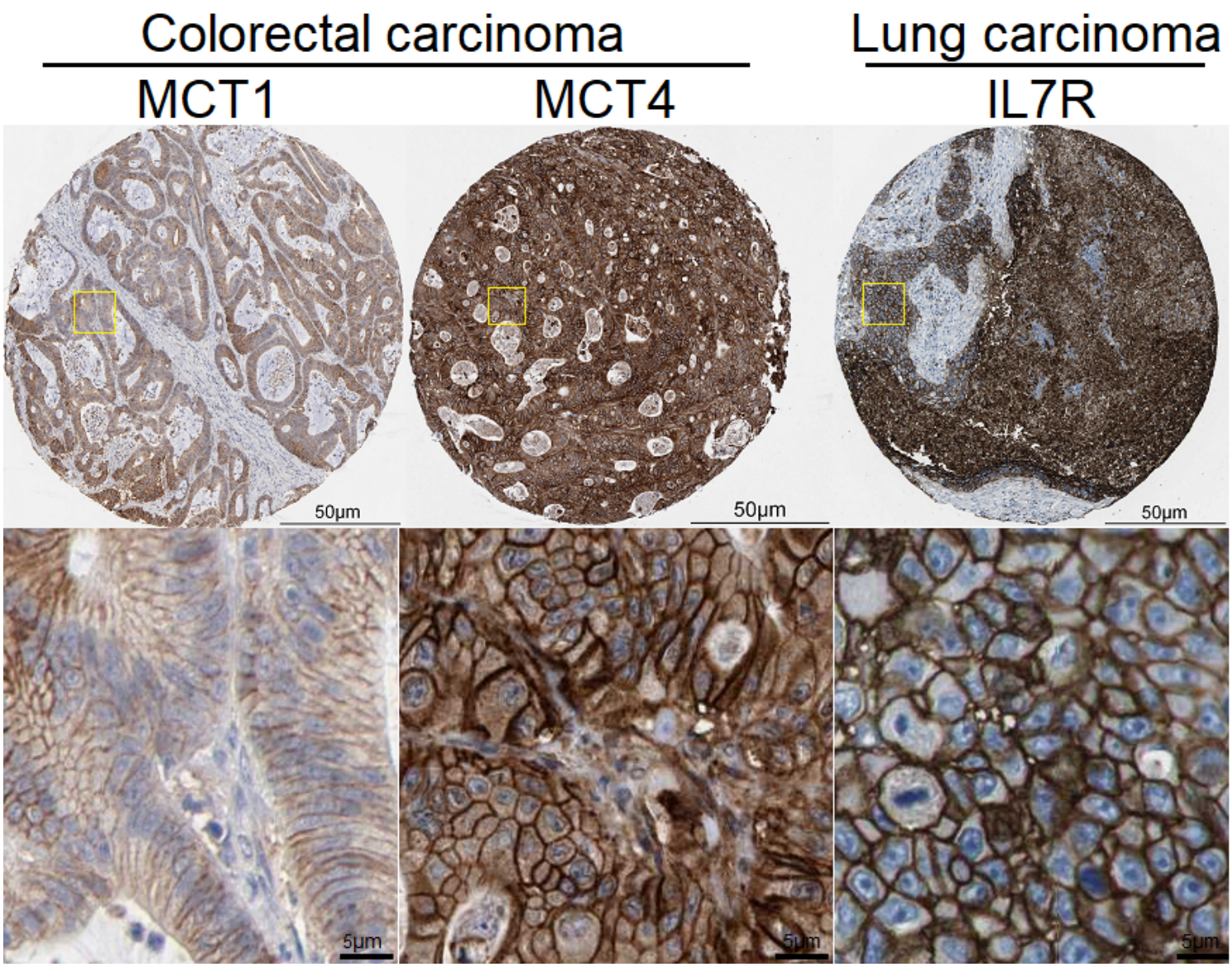

Figure 2

The positive control set up for immunohistochemical detection of MCT1, MCT4 and IL7R. Notably, here the lung cancer referred to was, actually, lung squamous cell carcinoma. The original magnification was 50 fold; magnification of the field of interest where boxed in yellow was 400 fold.

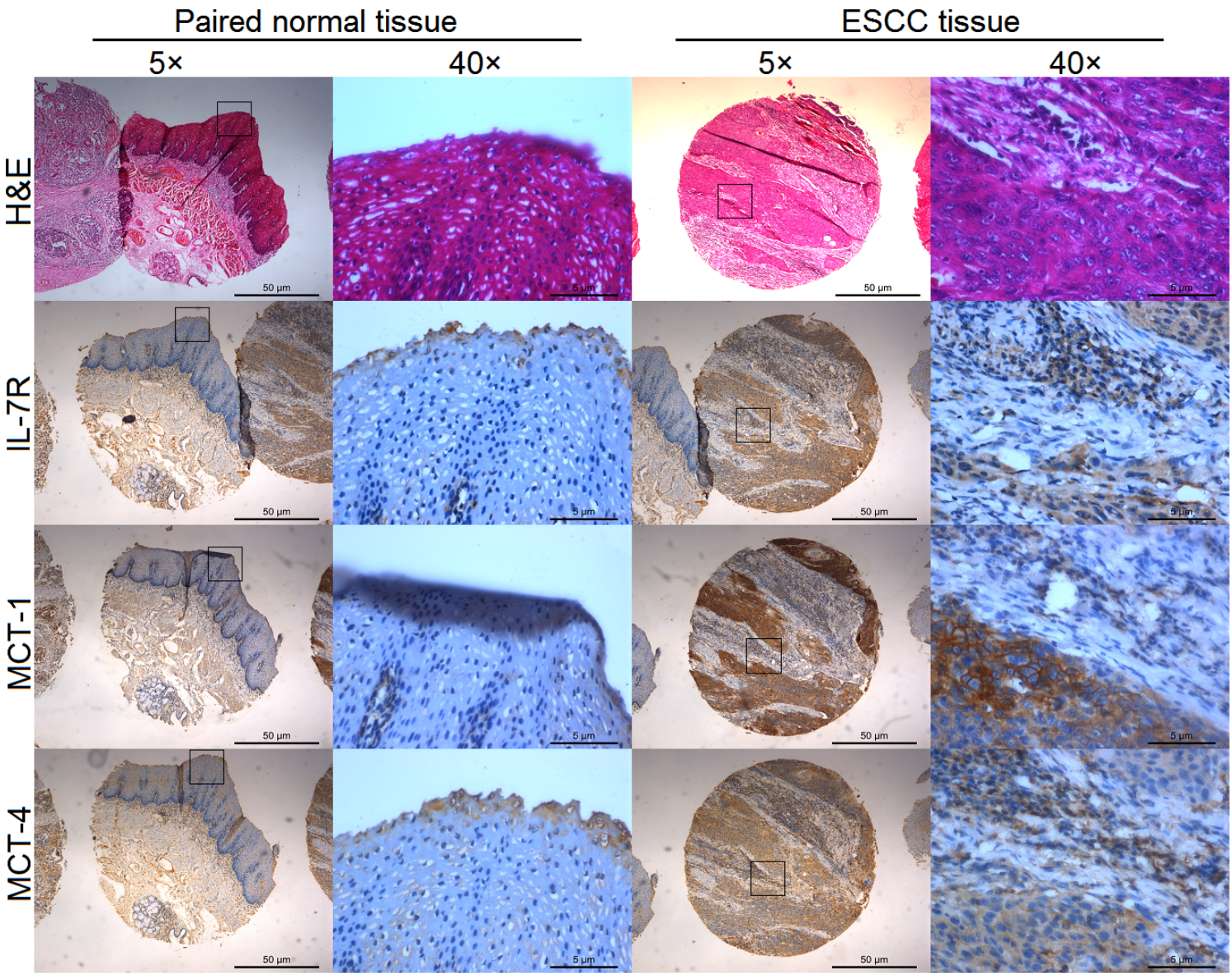

Figure 3

Immunohistochemical characteristics of MCT1, MCT4 and IL7R using the serial sections cut from the same tissue blocks of ESCC and its paired normal control. Distribution of ESCC cells expressing MCT1, MCT4 and IL7R was mainly restricted to stromal compartment. The original magnification was 50 fold; magnification of the field of interest was 400 fold. H&E, hematoxylin-eosin.

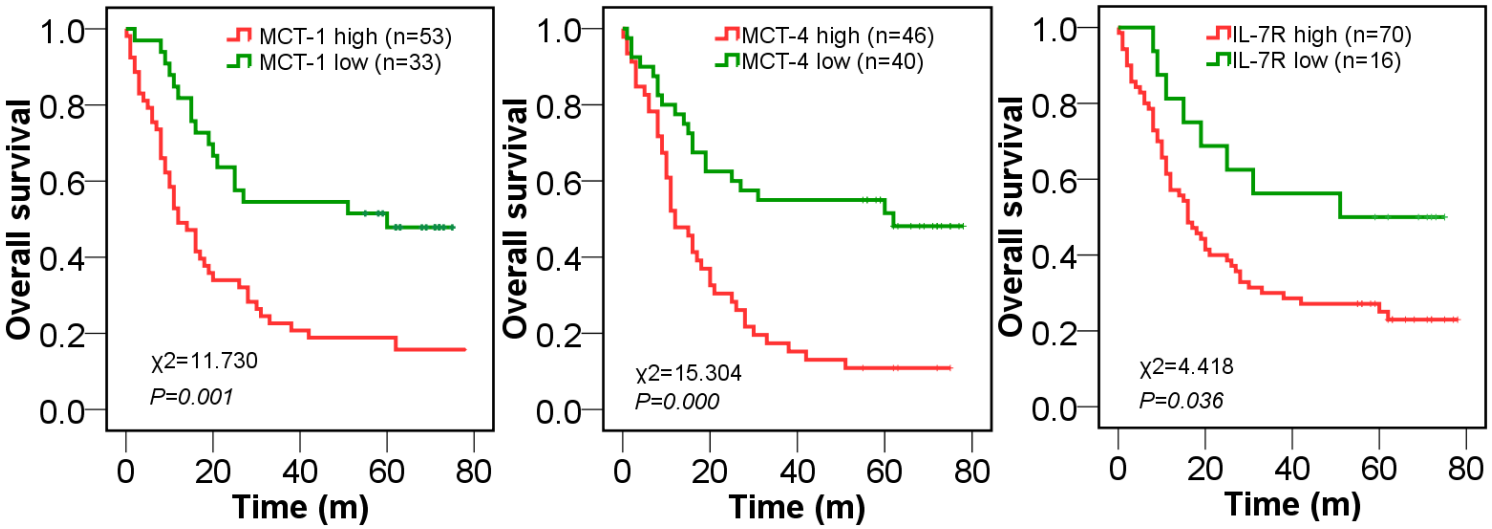

Figure 4

Prognostic significance of MCT1, MCT4 and IL7R expression in ESCC. Log-rank test was applied to analyze the statistical difference between high and low expression of MCT1, MCT4 and IL7R.