CONTROVERSIES IN IMMUNOHISTOCHEMISTRY IN RENAL CELL TUMORS

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Most of the renal cortical tumors can be easily classified on hematoxylin and eosin evaluation alone. However, there are some cases that need the support of immunohistochemistry and other ancillary tests for their proper classification. Such help is particularly important for the pathologists working in laboratories handling relatively low volumes of renal tumor specimens. The need for good, reliable immunohistochemical stains has also been progressively growing in the recent years, with pathologists increasingly being asked to render diagnosis on limited material (needle core biopsies and fine-needle aspirates).

Over the years, a large number of immunohistochemical markers have been reported to show remarkable sensitivity and specificity in discriminating between different renal cortical tumors. After the initial excitement created by the first few publications, very often the subsequent larger studies on wider range of tumors water down the initial claims. One of the common reasons for the initial “great” versus subsequent “not so great” results is that in most instances, the initial studies are performed on tumors that are morphologically typical representatives of a tumor type. As a matter of fact, in the day-to-day pathology practice, such cases hardly ever need immunohistochemical support for the diagnosis. When the same antibodies are used on tumors that are morphologically somewhat diverse and atypical, and that really need immunohistochemical support for the diagnosis, the results may be disappointing. As long as the original and subsequent investigators stick to the “factualness” of their observations, controversies will be very common. However, facts are facts; specific antibodies mark the tumors as reported in the initial publications, as well as in the subsequent papers. Only that, the authors in initial publications often did not investigate the tumors from the perspective that the subsequent authors did. And, non-acceptance of these simple facts makes a CONTROVERSY!

While none of them can be regarded as 100% sensitive and specific, in spite of the controversies, a number of antibodies are still useful in the differential diagnosis of renal cell tumors; some more so than others. A few of these are listed and discussed; the list should not be considered all inclusive.

Carbonic anhydrase-IX (CA-IX): It is a down-stream marker in the Hypoxia-Inducible Factor (HIF) pathway. HIF is generated in most body cells as a routine. However, in the presence of normal oxygen tension, it immediately gets hydroxylated. Hydroxylated HIF undergoes very quick proteosomic degradation in the presence of normal pVHL. Whenever there is tissue hypoxia, HIF does not get adequately hydroxylated; non-hydroxylated HIF cannot be degraded and is therefore over-expressed. In another scenario, at normal oxygen tension even when HIF gets hydroxylated, and normal pVHL is not present (as is usual in VHL syndrome, and very
common in sporadic clear cell RCCs), the hydroxylated HIF does not get degraded. Thus, it gets over-expressed and activates multiple down-stream proteins, including CA-IX.

CA-IX is a trans-membrane protein; therefore, the immunostain is primarily localized to the cell membranes. CA-IX is expressed in overwhelming majority (84-100%) of clear cell RCCs in a diffuse membranous pattern. Its expression has been shown to be retained in metastases from clear cell RCC. The staining is diffuse, irrespective of the nuclear grade of the tumor, although an occasional high grade tumor may show somewhat decreased staining intensity. Unlike any other antibody used for clear cell RCC, CA-IX expression is retained even in sarcomatoid areas in the overwhelming majority of sarcomatoid clear cell RCCs. The antibody is quite useful in the diagnosis of clear cell RCC even on limited material (needle core biopsies and cytological material). Although some pathologists are still concerned about its specificity (see below), the antibody (clone g250) is already being used in clinical trials by radiologists in the diagnosis of clear cell RCC at both the primary and metastatic sites. A recent multicentric phase 3 trial reported a specificity and sensitivity of greater than 86% each, in radiologic scans using a radio-labeled iodine and CA-IX compound in classifying renal tumors as clear cell RCC.

CA-IX also shows diffuse membranous immunoreactivity in clear cell papillary RCC, albeit qualitatively a little different from that seen in clear cell RCC. Positive staining has also been reported in bladder urothelial carcinoma, papillary RCC, and a number of other non-renal tumors. However, to the best of our knowledge, even when reported as positive with diffuse staining in only a small proportion of these positive tumors, none of these was reported to show expression in almost 100% of the tumor cells (as is characteristic in clear cell RCC). Such immunoreactivity likely is not related to the absence of pVHL in these tumors, but to the areas of tissue hypoxia. In most tumors other than clear cell RCC, when immunoreactivity is present, it is more prominent around the areas of tumor necrosis, and additionally in the case of papillary RCCs, close to the tips of papillae. Nevertheless, no immunostain can be used as a solitary marker in histo- or cyto-pathological diagnosis. Most of the non-clear cell RCC tumors with some CA-IX positivity will also show immunoreactivity to other markers that are appropriate for those specific tumors. In our experience, only VHL-related syndromic or sporadic tumors (e.g., clear cell papillary cystadenomas of the epididymis, cerebellar hemangioendotheliomas, etc.) show reactivity for CA-IX akin to that seen in clear cell RCC.

Another practical caveat in interpreting CA-IX staining is that in limited material (core biopsies, FNAs) that shows extensive tumor necrosis, positivity may be misleading. Most of the positive staining areas can represent peri-necrotic, hypoxic regions in the tumors. Therefore, interpretation of such biopsies requires great caution.

Overall, in our opinion, CA-IX is among the best available antibodies for recognizing clear cell RCC; primary, metastatic, high-grade, sarcomatoid, or on limited material.

**PAX 8/2:**
Pax8 and Pax2 are members of the paired box family of transcription factors and are very important in the fetal development of the central nervous system, eye, kidney, thyroid gland, and organs of the mesonephric (wolffian) and müllerian duct origin. They show nuclear expression in overwhelming majority of the tumors of renal cell origin, and are, therefore, very useful markers for establishing a renal cell derivation in both the primary and metastatic settings. Pax8 appears to be a more sensitive marker than Pax2, although only Pax2 may be expressed in very rare instances.

While evaluating staining for Pax markers, one always needs to remember that both Pax8 and Pax2 are not renal cell-specific, and are known to be expressed in a number of other tumors, including many tumors of the female genital tract, thyroid, a small proportion of upper urinary tract urothelial carcinoma, rare abdominal mesotheliomas, etc. At the same time, both may be absent in some tumors of renal origin, particularly some high grade tumors like collecting duct carcinoma, sarcomatoid RCCs, and tumors with rhabdoid features, as well as many chromophobe RCCs and renal oncocytesmas.

Thus, interpretation of staining results on Pax 8 or 2 needs to be performed in conjunction with other appropriate stains. Neither the presence of staining in isolation is definitive of renal cell origin, nor does absence of staining completely exclude such origin.

Renal Cell Carcinoma marker (RCCMa):

RCCMa, which is normally expressed in the proximal renal tubules, has been in use as a marker of renal cell origin for many years now. However, it shows sensitivity only in the range of 27-90 percent among primary renal tumors. At the same time the antibody labels a number of other tumors including lung adenocarcinoma, hepatocellular carcinoma, breast lobular carcinoma, ovarian clear cell carcinoma, parathyroid carcinoma and many others. In a more recent publication by Sharma et al, the antibody was shown to label greater than 40% of non-renal tumors with papillary architecture from various sites.

Given the availability of some better antibodies for the diagnosis of renal cell origin, RCCMa appears to have very limited clinical value now.

CK7:

CK7, a commonly utilized antibody in most surgical pathology laboratories, has multiple utilities in the differentiation of renal cell tumors. CK7 is usually diffusely expressed in type 1 papillary RCC, classical chromophobe RCC and clear cell-papillary RCC. At the 2012 Vancouver ISUP consensus conference, a majority of participants voted for performing immunohistochemical
stains (including CK7) to diagnose clear cell-papillary RCC, in addition to taking the morphologic features into consideration.

CK7 is usually negative or very focally positive in acquired cystic disease-associated RCC, even when it shows a prominent papillary architecture. CK7 is also generally negative in clear cell RCC, although it may show focal positivity in these tumors around cystic areas. Renal oncocytomas are either negative, or show only rare immunoreactive cells. While it may be useful in distinction of oncocytoma from classical chromophobe RCC, in general CK7 is not very useful in distinction of oncocytoma from eosinophilic variant of chromophobe. Such eosinophilic variants only rarely show diffuse CK7 positivity; the labeling in eosinophilic chromophobes is more often only focal or patchy. Similarly, type 2 papillary RCCs also very often show only limited staining with CK7, unlike the diffuse pattern in type 1 tumors.

CK7 remains a useful antibody in the differentiation of many renal tumors. However, too much dependence of the staining results is nor warranted in the distinction of renal oncocytoma from eosinophilic variant of chromophobe RCC, or type 2 papillary RCC from its mimics.

**CD10:**

CD10 has been used for many years in the distinction of clear cell RCC from other tumors, particularly in metastatic setting. Only membranous staining can be considered as compatible with clear cell RCC, in an appropriate clinicopathological scenario. On the other hand, cytoplasmic positivity for CD10 is seen in a large number of non-renal and renal tumors. Additionally, membranous immunoreactivity for CD10 is not limited to clear cell RCC, and may be present in papillary RCC, colorectal, pancreatic, prostatic, or some vascular tumors as well.

CD10 cannot be used in isolation to diagnose clear cell RCC, either in primary or metastatic setting. Given that better markers for renal cell (like Pax8/2) or clear cell RCC (like CA-IX) origin are now available, the dependence on CD10 may be on the wane.

**Others:**

The search for reliable immunohistochemical stains that can consistently distinguish between oncocytic neoplasms (renal oncocytoma, eosinophilic chromophobe RCC, low-grade oncocytic unclassified RCC) has been long and frustrating. A number of immunostains have been proposed in this distinction, but in the long run, none has proven to be as efficient as claimed in the initial publications. The likely reasons for that are discussed above. Multiple antibodies used in this distinction, over the past 1½ to 2 decades include, anti-mitochondrial antibodies, E-cadherin, Kidney-specific cadherin, S100A1, EpCam, etc. Kangai 1 (KAI1/CD82), product of metastasis
Suppressor gene, in a few recent publications appears to have some potential. But, more evidence is needed yet.

References:


Controversies in immuno-histochemistry in renal cell tumors

Satish K. Tickoo
Controversy

- Controversy: Merriam-Webster- “discussion marked especially by the expression of opposing views”

- So… on controversial issues- what is true
  - Most often all points of view; generally the truth, but often not “the whole truth” and “nothing but the truth”
  - Example; 20° F temperature in NYC- both cold and “quite warm”

- Therefore, controversy very often the creation of one’s unidirectional thought processes
Immunohistochemistry in renal tumors

- Most stains “help” in tumors where no help is really needed
- Some antibodies- put in the controversy list- may actually be useful if used appropriately
  - Carbonic Anhydrase-IX (CA-IX)
  - Pax-8/2
  - CK7
  - CD10
  - RCC Ma
Carbonic Anhydrase-IX (CA-IX)

- CA-IX, a trans-membranous enzyme/protein with functions to regulate intracellular and extracellular pH
- Expression induced by hypoxic/pseudohypoxic conditions; downstream to HIF protein: overexpression related to hypoxia or lack of pVHL
- Diffuse membranous positivity in 84-100% clear cell RCCs

CA-IX; Pros……

- Diffuse membranous + in clear cell RCCs
  - Both primary and metastatic (84 vs. 89%)
    - (Tickoo et al. USCAP 2007)
  - Even in high-grade areas with solid, pseudo-papillary, papillary areas (3+ expression; 88 vs. 95%)
  - 3/4ths of sarcomatoid clear cells in sarcomatoid areas (3+ expression; 73 vs. 86%)
    - (Tickoo et al. J Urol. 2007;177:1224)
CA-IX; Pros……

- Highly useful on limited material
  - Biopsies
  - Cell blocks (Personal experience)

- CA-IX (g250) (chimeric$^{124}$I-cG250) in phase 2 and 3 trials for radio-diagnosis of clear cell RCC
Positive in non-renal tumors
- More concentrated around necrosis; cells away from necrosis, mostly negative

Positive in urothelial carcinomas
- Almost never 100% or close to 100% cells (26% HIF-1, 3+; Tickoo et al. BJU International 2010;107:844; 14-18% CA-IX, 3+; Donato et al. 2011 Histopathology;59:1229-- +3= >50%)
- Usually CK7/CK20/HMWCK/p63 positive

Reportedly positive in papillary RCC
  - Around necrosis and papillary tips; never anywhere close to 100% cells in our experience
  - CK7/AMACR positive
CA-IX; Cons……

- Positive in translocation-associated RCC and HLRCC-associated carcinomas
  - Usually absent or patchy staining
- Clear cell papillary RCC
  - Always positive
CA-IX

- To date- the best marker in our hands/opinion for clear cell RCC, particularly when used in combination with H&E and other markers
Pax 8/2; Pros……

- Overwhelming majority, but not all, of renal tumors positive
- Clean nuclear stain
- In most cases both positive, but rarely only one and not the other (Pax 8 generally > Pax 2)
- If clinically renal tumor a consideration, positivity strongly supports a renal origin
Pax8/2; Cons ............

- Tumors from other organs positive (Female genital tract, thyroid, etc.)
- May not be positive in high grade tumors of renal cortical origin
- Positive in a small proportion of upper urinary tract urothelial carcinomas

<table>
<thead>
<tr>
<th>Organ</th>
<th>Diagnoses</th>
<th>Positive/total cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kidney</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RCC, clear cell</td>
<td>90/95 (95%)</td>
<td></td>
</tr>
<tr>
<td>RCC, papillary&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38/38 (100%)</td>
<td></td>
</tr>
<tr>
<td>RCC, chromophobe</td>
<td>22/25 (88%)</td>
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</tr>
<tr>
<td>RCC, collecting duct</td>
<td>5/7 (71%)</td>
<td></td>
</tr>
<tr>
<td>RCC, sarcomatoid</td>
<td>4/9 (44%)</td>
<td></td>
</tr>
<tr>
<td>RCC, rhabdoid</td>
<td>0/4 (0%)</td>
<td></td>
</tr>
<tr>
<td>RCC, mucinous tubular</td>
<td>6/6 (100%)</td>
<td></td>
</tr>
<tr>
<td>RCC, ACK-related</td>
<td>8/8 (100%)</td>
<td></td>
</tr>
<tr>
<td>RCC, translocation</td>
<td>1/2 (50%)</td>
<td></td>
</tr>
<tr>
<td>RCC, tubulocystic</td>
<td>1/1 (100%)</td>
<td></td>
</tr>
<tr>
<td>Metanephric neoplasms</td>
<td>3/3 (100%)</td>
<td></td>
</tr>
<tr>
<td>Mixed epithelial and stromal tumors</td>
<td>4/4 (100%)</td>
<td></td>
</tr>
<tr>
<td>Cystic nephroma</td>
<td>4/4 (100%)</td>
<td></td>
</tr>
<tr>
<td>Urothelial carcinoma</td>
<td>0/13 (0%)</td>
<td></td>
</tr>
<tr>
<td>Oncocytoma</td>
<td>8/13 (61%)</td>
<td></td>
</tr>
<tr>
<td>Angiomyolipoma</td>
<td>0/8 (0%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>194/240 (89%)</td>
<td></td>
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<tr>
<td><strong>Female genital tract</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
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</tr>
<tr>
<td>Endometrioid carcinoma</td>
<td>72/77 (93%)</td>
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</tr>
<tr>
<td>Endometrial polyp</td>
<td>8/8 (100%)</td>
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<tr>
<td>Serous carcinoma</td>
<td>101/102 (99%)</td>
<td></td>
</tr>
<tr>
<td>Serous adenoma/borderline tumors</td>
<td>22/23 (95%)</td>
<td></td>
</tr>
<tr>
<td>Mucinous carcinoma</td>
<td>4/10 (40%)</td>
<td></td>
</tr>
<tr>
<td>Mucinous adenoma/borderline tumors</td>
<td>3/13 (23%)</td>
<td></td>
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<tr>
<td>Clear cell carcinoma</td>
<td>16/16 (100%)</td>
<td></td>
</tr>
<tr>
<td>Other types&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12/18 (66%)</td>
<td></td>
</tr>
<tr>
<td>Cervical squamous cell carcinoma</td>
<td>0/9 (0%)</td>
<td></td>
</tr>
</tbody>
</table>

Seminal vesicles: 27/27 (100%)
Epididymis: 17/17 (100%)

*Ozcan et al. Modern Pathol. 2011;24:751*
<table>
<thead>
<tr>
<th>Renal Cell Carcinoma Type</th>
<th>PAX2*</th>
<th></th>
<th>PAX8†</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>Positive Cases (%)</td>
<td>n</td>
</tr>
<tr>
<td>Clear cell RCC, primary</td>
<td>330</td>
<td>288 (87)</td>
<td>248</td>
</tr>
<tr>
<td>Papillary RCC, primary</td>
<td>179</td>
<td>108 (60)</td>
<td>59</td>
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<tr>
<td>Chromophobe RCC, primary</td>
<td>117</td>
<td>19 (16)</td>
<td>43</td>
</tr>
<tr>
<td>Collecting duct RCC, primary</td>
<td>13</td>
<td>5 (38)</td>
<td>30</td>
</tr>
<tr>
<td>Tubulocystic RCC, primary</td>
<td>33</td>
<td>13 (39)</td>
<td>1</td>
</tr>
<tr>
<td>Mucinous tubular and spindle cell RCC, primary</td>
<td>7</td>
<td>6 (86)</td>
<td>6</td>
</tr>
<tr>
<td>Xp11 translocation RCC, primary</td>
<td>36</td>
<td>14 (39)</td>
<td>2</td>
</tr>
<tr>
<td>Sarcomatoid RCC, primary</td>
<td>7</td>
<td>0 (0)</td>
<td>18</td>
</tr>
<tr>
<td>Clear cell RCC, metastatic</td>
<td>323</td>
<td>202 (63)</td>
<td>329</td>
</tr>
<tr>
<td>Papillary RCC, metastatic</td>
<td>8</td>
<td>6 (75)</td>
<td>13</td>
</tr>
<tr>
<td>Sarcomatoid RCC, metastatic</td>
<td>5</td>
<td>0 (0)</td>
<td>8</td>
</tr>
<tr>
<td>Urothelial carcinoma, upper urinary tract</td>
<td>47</td>
<td>0 (0)</td>
<td>64</td>
</tr>
</tbody>
</table>

RCC indicates renal cell carcinoma.

*From references.18,20,22–33

†From references.30,32,33,43–47
Pax 8/2

- Positivity cannot be used in isolation as diagnostic of renal origin
- Absent staining does not exclude renal origin, particularly so in high grade tumors
RCCMa: Pros and Cons

- Renal Cell Carcinoma marker (RCCMa) an antibody for RCC with reported sensitivity and specificity of approximately 75%
- Reasonable, but not great sensitivity and specificity
- Positive in a number of non-renal primary tumors
  - lung adenocarcinomas
  - hepatocellular carcinomas
  - lobular carcinoma breast
  - parathyroid carcinomas
  - ovarian clear cell carcinomas
  - testicular and extra-gonadal seminomas, yolk sac tumor
<table>
<thead>
<tr>
<th>Histologic Subtype</th>
<th>Positive</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classic clear cell</td>
<td>46/64</td>
<td>72</td>
</tr>
<tr>
<td>Papillary renal cell</td>
<td>40/42</td>
<td>95</td>
</tr>
<tr>
<td>Unclassified</td>
<td>33/38</td>
<td>85</td>
</tr>
<tr>
<td>Sarcomatoid</td>
<td>6/30</td>
<td>20</td>
</tr>
<tr>
<td>Oncocytic</td>
<td>18/24</td>
<td>75</td>
</tr>
<tr>
<td>Chromophobe</td>
<td>20/22</td>
<td>91</td>
</tr>
<tr>
<td>Metatastatic</td>
<td>10/23</td>
<td>40</td>
</tr>
<tr>
<td>Total (all types)</td>
<td>173/241</td>
<td>72</td>
</tr>
</tbody>
</table>
RCC Ma: Cons...

- RCCMa positive in 28/66 (42%) of non-renal papillary neoplasms, including:
  - 9/9 (100%) papillary thyroid carcinomas
  - 8/10 (80%) ovarian papillary serous carcinomas
  - 4/9 (44%) uterine papillary serous carcinomas
  - 1/10 (10%) papillary urothelial carcinomas
  - 1/2 (50%) intraductal papillary mucinous carcinomas of the pancreas
  - 3/3 (100%) choroid plexus papillomas
  - 1/1 (100%) pituitary adenoma with papillary features
  - 1/2 (50%) lung adenocarcinomas with papillary features

CK7

- Is a commonly used antibody in most pathology labs
- Usually positive in papillary and chromophobe RCC
- Mostly negative in clear cell RCC
- Very focally positive in renal oncocytoma
CK7; Pros……

- Very reliable antibody for clear cell papillary RCC- diagnostic utility (ISUP 2012)
- Quite reliable for papillary RCC, particularly type 1
- Often diffusely positive in classical chromophobe RCC
- Variably positive in collecting duct carcinoma
CK7; Cons........

- Clear cell RCCs with cystic components, often positive
  - *(Fine et al. 2012, USCAP)*

- Eosinophilic chromophobe RCC, often very focal or negative
  - Not of much use in distinction from oncocytoma

- Type 2 papillary RCC, often negative
Papillary

Chromophobe

Oncocytoma

Oncocytoma
CK7

- Use and interpret with caution in tumors where it is needed the most (oncocytoma vs. eosinophilic chromophobe; high grade papillary)
CD10

- **Common Acute Lymphoblastic Leukemia Antigen (CALLA)**
- Labels clear cell RCC in a membranous pattern (approximately 80%)
  - (Liu et al. Arch Pathol Lab Med. 2007;131:1290; Tickoo et al. 2007, USCAP)
- Labels papillary RCC
  - Usually along the apical membrane
- **Variable expression apical/membranous/cytoplasmic in chromophobe RCC and oncocytoma (0-35%)**
CD10; Pros……

- In metastatic setting, may differentiate from clear cell adenocarcinoma of GYN tract
CD10: Cons.....

- Apical surface only: well-differentiated colonic, pancreatic and prostatic adenocarcinoma
- Canalicular pattern in HCC may mimic membranous positivity, particularly in limited tissue
- Diffuse cytoplasmic or membranous/Golgi patterns: poorly differentiated adenocarcinoma, melanoma, urothelial carcinoma, renal cell carcinoma, endometrial stromal sarcoma
  - (Am J Clin Pathol 2000;113:374)
- Epithelioid hemangioendothelioma can be strong/diffuse CD10+ and mimic metastatic renal cell carcinoma
  - (Arch Pathol Lab Med 2009;133:1965)
- Solid and pseudopapillary pancreatic tumor is CD10+
  - (Am J Surg Pathol 2000;24:1361)
CD10

- To support the diagnosis of clear cell RCC, positivity has to be membranous; cytoplasmic positivity is seen in a number of other tumors
- Cannot depend on CD10 alone
Anti-Mito, E-Cad, Ksp-Cad, S100A1, KAI1…

- Multiple markers claimed to distinguish between oncocytoma and chromophobe RCC
- Initial publications raise expectations, but ultimately none proven to be great
  - Kidney specific-Cadherin: “The findings argue against the use of Ksp-cad in differentiating chromophobe renal cell carcinoma and renal oncocytomas”
    
    (Kuehn et al. Am J Surg Pathol. 2007;31:1528)

- Currently, KAI1 being touted as potentially useful

Conclusions

- “When a thing ceases to be a subject of controversy, it ceases to be a subject of interest”. William Hazlitt
- “In a controversy the instant we feel anger we have already ceased striving for the truth, and have begun striving for ourselves”. Buddha