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Roles of the Pathologist in Evaluating Surrogate Markers for Medical Therapy in Adrenocortical Carcinoma

Hironobu Sasano · Fumitoshi Satoh · Yasuhiro Nakamura

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Abstract Adrenocortical carcinoma is a rare malignancy. Medical treatment including op'DDD or mitotane with or without platinum-based cytotoxic chemotherapy is frequently administered to the patients in an adjuvant setting following surgery or in advanced disease, because of aggressive clinical behavior in some cases. Potential roles of pathologists in determining the clinical algorithm of medical therapy are histopathological confirmation of adrenocortical carcinoma, both malignant features using the criteria of Weiss and adrenocortical origin applying immunohistochemistry of steroid factor-1 (SF-1); providing the relevant pathological information to determine the precise pathological stage in individual patients; and providing the accurate Ki67 labeling index of the patients. There are no established pathological surrogate markers for response to mitotane or op' DDD therapy available at this juncture but as in other malignancies, Ki67 LI could provide information as to the potential clinical response to platinum-based cytotoxic chemotherapy. Epidermal growth factor receptor (EGFR) is significantly overexpressed in adrenocortical carcinoma but the absence of gene mutations could limit the therapeutic application of anti-EGFR antibody and/or EGFR tyrosine kinase inhibitor in the patients.

Keywords Adrenal · Pathology · Carcinoma · Immunohistochemistry · Mitotane · Chemotherapy · EGFR · Surrogate marker

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H. Sasano (✉) · Y. Nakamura
Department of Pathology, Tohoku University School of Medicine, 2-1 Seiryou-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: hsasano@patholo2.med.tohoku.ac.jp

F. Satoh
Department of Medicine, Tohoku University School of Medicine, 2-1 Seiryou-machi, Aoba-ku, Sendai 980-8575, Japan

Introduction

Adrenocortical carcinoma is a relatively rare malignancy that demonstrates aggressive clinical behavior in some patients resulting in dismal clinical outcome. There are bimodal peaks in its incidence, at relatively young and elderly ages. Therefore, the clinical management can be sometimes difficult and challenging and clinicians ask for comments other than histopathological diagnosis from the pathologists regarding the management of the patients. In these circumstances, three of the most common questions asked of surgical pathologists are summarized as follows:

1. Whether the adrenocortical mass represents a benign adenoma or a malignant carcinoma? On what basis can the diagnosis be reached?
2. Whether the lesion is of adrenocortical origin or not? In cases of advanced malignancy with primary unknown or large retroperitoneal masses.
3. Whether the patient could respond to the medical therapy or not? In cases of proven adrenocortical carcinoma with adjuvant therapy settings after the surgery or in advanced stages with multiple distant metastasis.

Malignancy or not in resected adrenocortical neoplasm?

In the differential diagnosis between adrenocortical adenoma and carcinoma, the histopathological criteria of Weiss [1, 2], possibly in conjunction with some of recently reported modifications [3–5], still represents the gold standard of reliable differentiation between adult adrenocortical adenoma and carcinoma in surgically resected adrenocortical neoplasms, despite the marked recent evolution or improvement of various molecular diagnostics. Therefore, it is important for pathologists to be very familiar with these criteria because

interpretation of these nine factors is essentially straightforward but could be subjective, resulting in inter-observer differences, as many clinicians have grumbled. However, it is also important for clinicians to be well aware of the validity and utility as well as limitation of this scoring system when they receive the surgical pathology report of a patient with adrenocortical neoplasm.

Adrenocortical origin or not?

When diagnosing whether the lesions are of adrenocortical origin or not, a detailed histopathological evaluation of hematoxylin-eosin-stained tissue slides is still sufficient in the majority of the cases. However, it is also true that some malignancies such as renal cell carcinoma, especially clear cell type, malignant melanoma, hepatocellular carcinoma, large cell type neuroendocrine carcinoma of the lung and others, could pose diagnostic difficulties in pathological evaluation of biopsy or surgical materials. This differential diagnosis is clinically important in patients with advanced stages because of availability of target-specific therapy in other malignancies such as clear cell type renal cell carcinoma or melanoma. Immunohistochemical evaluation of markers relatively specific for adrenocortical parenchymal cells, such as inhibin alpha, calretinin, and steroid factor-1 (SF-1) [6–10], especially SF-1 [7, 8] could be certainly of value in this differential diagnosis. However, it is also important for pathologists to recognize that SF-1 immunohistochemistry is the most specific because, apart from the adrenal cortical tumors, only gonadotropin-producing pituitary adenoma and certain sex cord stromal cell tumors express this nuclear transcription factor in the nuclei [7]. However, its sensitivity is markedly influenced by pre-analytical factors such as fixation of the specimen.

Aderenocortical carcinoma, aggressive type or not? Response to therapy?

When pathologists are asked by clinicians, especially medical oncologists or endocrinologists about the clinical problem above, the situation is not as straightforward as in questions no. 1 and 2. The most important prognostic factor of patients with adrenocortical carcinoma is obviously the clinical and pathological stage [11, 12], and application of the staging system proposed by ENSAT could provide more accurate information as to the clinical outcome of the patients [12]. Therefore, for the sake of the patients, pathologists should also attempt to do their best to provide the necessary information toward accurate staging to the clinicians. However, it is also true that there are no other established histopathological or other parameters in carcinoma cells of predicting clinical outcome at this juncture, although some factors such as Ki67 LI and the combination of factors in Weiss criteria have been reported promising [13, 14]. In addition, since Terzolo et al. reported the value of op'DDD to the patients with adrenocortical carcinoma as an adjuvant therapy not only in improving their disease free survival but also prognosis itself [15], an increasing number of clinicians managing patients with adrenocortical carcinoma have been asking the pathologists to provide any information as to whether there are any histopathological, immunohistochemical, or molecular findings suggestive of response to the mitotane or op' DDD with or without chemotherapy in resected specimens of the tumor.

In this review, we will first cover the reported histopathological surrogate markers predicting the response to conventional therapy such as mitotane or op'DDD with or without platinum-based chemotherapy in patients with adrenocortical carcinoma. We will then report the potential surrogate markers to which target-specific therapy is being developed or has been employed in other human malignancies, reported to be detected in adrenocortical carcinoma.

Surrogate Markers of Conventional Medical Therapy in Adrenocortical Carcinoma

Op' DDD and mitotane combined with platinum based chemotherapy have been reported to yield objective responses but their impact upon actual prognosis of the patients still remains unestablished [16]. Cytotoxic chemotherapy regimens combining platinum with doxorubicin–etoposide or streptozotocin have been reported to yield highest objective response rates in patients with adrenocortical carcinoma [16]. Therefore, the administration of mitotane and cytotoxic chemotherapy is currently considered a well-established therapeutic regimen of the adrenocortical carcinoma patients with advanced-stage disease.

Potential pathological markers of response to platinum-based chemotherapy

In adjuvant settings, it was reported that those with a Ki67 labeling index of greater than 10 %, considered high risk patients expected to have aggressive clinical course, could benefit from this combined therapy following surgery of the primary tumor and those with less than 10 %, considered low to intermediate risk group, could receive only mitotane treatment as a postoperative adjuvant therapy [13, 14]. Thus, clinicians have recently asked pathologists to perform Ki67 immunohistochemistry and obtain the labeling index of the resected adrenocortical carcinoma specimens. However, it is also to be noted that the clinical studies based on this proposal of cutting off value of 10 % have been well and carefully executed but the major limitation is that the immunostaining and evaluation of Ki67 labeling index had not been centrally

performed and so many pre- and post-analytical factors of Ki67 evaluation appear to have compromised the results [17, 18]. Therefore, at this juncture, there are no doubts that postoperative course is definitely different between low/intermediate and high grade adrenocortical carcinoma patients and the latter could receive clinical benefits from being treated with mitotane and cytotoxic chemotherapy but Ki67 LI of 10 % is by no means the magic number and further investigations are required for further consolidation of this subclassification or stratification of the patients.

The same concept is also reported in NET or neuroendocrine tumor [19]. Among NET G3 or neuroendocrine carcinoma (NEC) with Ki67 LI of more than 20 %, those with more than 55 % of Ki67 LI had significantly worse clinical outcome but better response to platinum-based chemotherapy and those with less than 55 % had better clinical outcome but less response to the same chemotherapy [19]. Those results did indicate that it is also important for pathologists to standardize the evaluation of Ki67 LI in adrenocortical carcinoma in order to make maximum use of this marker identifying the proliferative cells in surgical pathology specimens and pivotal for clinicians to recognize that there has been no definitive magic number of Ki67 LI to definitively stratify patients following surgery in the adrenocortical carcinoma. Currently, clinical judgment could be more important that mere Ki67 LI in contrast to other malignancies such as breast or NET.

For the response to platinum-based chemotherapy in adrenocortical carcinoma patients, Ronchi et al. reported that the expression of excision repair cross complementing group 1 in the tumor cells could predict the response to cisplatinum therapy [20]. However, Malandrino [21] reported the absence of such a correlation and it also awaits further investigations for clarification.

Surrogate markers of response to mitotane or op'DDD treatment?

As noted above, mitotane is currently recommended as an adjuvant therapy essentially in all patients with adrenocortical carcinoma when clinically applicable. However, mitotane is associated with various side effects, some of them serious [22] and it then becomes important to discern the factors predicting the response to mitotane administration. Mitotane or op'DDD, an adrenolytic agent, has been used for a relatively long period of time, mostly in advanced adrenocortical carcinoma with various clinical response rates but at this juncture, there have been no established pathological or biological parameters in adrenocortical carcinoma cells or tissues which could predict the clinical response to mitotane, although Volante et al. reported the possible value of ribonucleotide reductase large subunit gene expression in the carcinoma cells as a surrogate marker for response to mitotane treatment [23] . Therefore, the reasonable response when asked by clinicians about the surrogate markers specific for mitotane treatment is that none are available at present, other than the fact that the lesion is an adrenocortical carcinoma.

Surrogate markers of potential target-specific therapy in adrenocortical carcinoma

Target-specific therapy has been more widely administered in an increasing number of human malignancies, and the roles of surgical pathologists are becoming more important in identifying the specific surrogate markers of these targets in surgical pathology specimens, whether biopsies or resections. There are no established target-specific therapies available in the medical treatment of adrenocortical carcinoma patients at present but many basic, translational, and clinical research studies, including clinical trials of some agents, are being conducted and it is not entirely surprising that agent(s) based upon these cutting edge studies will be approved and incorporated into clinical practice in the very near future.

The development of target-specific therapy in adrenocortical carcinoma has been mostly performed by the following two different approaches. The first is to attempt to administer the agents which have been approved in other human malignancies to the patients with adrenocortical carcinoma with pathological exploration of corresponding surrogate markers. The other is to identify the potential targets by using genome-wide screening or other relevant methods. We will cover the first approach in this mini-review because a considerable number of relevant reviews have been reported in the latter.

The Application of Existing Target-Specific Therapy to Medical Treatment of Adrenocortical Patients

Epidermal Growth Factor Receptor (EGFR)

Nakamura was the first to explore the ten well established or potential surrogate markers of target-specific therapies, located in the Ras/extracellular signal-regulated kinase and phosphatidylinositol-3 kinase/Akt pathways in 41 cases of surgical pathology specimens of adrenocortical carcinoma using immunohistochemistry and compared the results with those in 54 adrenocortical adenomas [24]. Results of their analysis demonstrated that only EGFR was more significantly overexpressed in carcinoma compared to adenoma.

EGFR was firstly demonstrated to be overexpressed in adrenocortical carcinoma in 1994 by Sasano et al. [25]. Therefore, it is interesting to note that monoclonal anti-EGFR antibody and/or EGFR tyrosine kinase inhibitor could provide therapeutic benefits to the patients of adrenocortical carcinoma as in lung or colon cancer cases. However, Adam et al. reported in 2010 that the analysis of 169 cases of

adrenocortical carcinoma cases demonstrated no significant correlation with clinical outcome and absence of point mutations of EGGR gene, which could serve as important surrogate markers in EGFR tyrosine kinase inhibitors [26]. Very recently, Zhang et al. also reported the overexpression of the EGFR protein and polysomy of chromosome 7 in which the EGFR gene is located but the absence of any of 29 specific mutations in exons 18 to 21 in adrenocortical carcinoma patients [27]. These results all indicated some roles of EGFR-mediated intracellular signaling pathways in adrenocortical carcinoma cells but it is considered highly unlikely that the current therapies aimed at EGFR pathways could provide therapeutic benefits to patients with adrenocortical carcinoma. The identification of EGFR overexpression could rather serve as a potential auxiliary or confirmatory marker of discerning malignancy in adrenocortical neoplasm [26].

mTOR inihibitors

Insulin-like growth factor (IGF) system has been demonstrated to play important roles in the biology of adrenocortical cells by the results of various genome-wide screening studies [28]. However, it is true that specific agents inhibiting this IGF-related pathway are promising but not available for clinical practice at this juncture. Mammalian target of rapamycin (mTOR) inhibitor, which has been used in the treatment of NET, breast cancer, and clear cell type renal cell carcinoma, was reported to be administered to the advanced stages of adrenocortical carcinoma patients despite little clinical improvement [29]. In addition, De Martino et al. very recently evaluated the expression of mTOR as well as S6K1 and 4 EBP1, both of which are important downstream factors in the mTOR pathway, in adrenocortical carcinoma patients and reported that subsets of the patients did demonstrate the activation of mTOR pathways in carcinoma cells, which could lead to the potential efficacy of mTOR inhibitor [30]. However, the major limitation of this research is that the identification of S6K1 and 4EBP1, even in phosphorylated forms, does by no means serve as an established surrogate marker of mTOR inhibitor efficacy in any human malignancy, although immunohistochemical overexpression of pmTOR, pS6K1, and p4EBP1 does suggest the presence of mTOR activation in the cells. Therefore, it is important for pathologists to inform clinicians of this, but to indicate that this testing is still at the stage of research or academic exercise if they ask for immunohistochemical evaluation of mTOR pathway-related factors in surgical pathology specimens of adrenocortical carcinoma.

Conclusion

Unless new therapeutic agents are being administered in a clinical trial, the current roles of surgical pathologists in providing information to help with decisions on the medical therapy of patients with adrenocortical carcinoma patients are as follows: (1) accurate pathology diagnosis of adrenocortical carcinoma, (2) determination of adrenocortical origin if applicable, (3) providing the relevant findings for the accurate determination of TNM stages, and (4) evaluation of Ki67 labeling index. However, one should follow the development of novel target-specific therapeutic agents for patients with adrenocortical carcinoma with companion diagnostic means of identifying their surrogate markers.

References

1. Weiss LM. Comparable histologic study of 43 metastasizing and nonmetastasizing adrenocortical tumors. Am J Surg Pathol 1984; 8: 163–169,
2. Weiss LM, Medeiros LJ, Vickery AL. Pathologic features of prognostic significance in adrenocortical carcinoma. Am J Surg Pathol. 1989;13:202–206,
3. Aubert S, Wacrenier A, Leroy X, Devos P, Carnaille B, Proyce C, Wemeau JL, Lecomte-Houcke M, Leteurte E. Weiss system revisited: a clinicopathologic and immunohistochemical study of 49 adrenocortical tumors. Am J Surg Pathol 2002; 26:1612–1219
4. Papotti M, Libè R, Duregon E, Volante M, Bertherat J, Tissier F. The Weiss score and beyond–histopathology for adrenocortical carcinoma.Horm Cancer 2011; 2:333–340
5. Duregon E, Fassina A, Volante M, Nesi G, Santi R, Gatti G, Cappellesso R, Dalino Ciaramella P, Ventura L, Gambacorta M, Dei Tos AP, Loli P, Mannelli M, Mantero F, Berruti A, Terzolo M, Papotti M. The reticulin algorithm for adrenocortical tumor diagnosis: a multicentric validation study on 245 unpublished cases. Am J Surg Pathol. 2013; 237:1433–1440
6. Sasano H. New approaches in human adrenocortical pathology: assessment of adrenocortical function in surgical specimen of human adrenal glands. Endocr Pathol 1992; 3:4–13
7. Sasano H. Shizawa S. Suzuki T. Takayama K. Fukaya T. Morohashi K. Nagura H. Transcription factor adrenal 4 binding protein as a marker of adrenocortical malignancy. Human Pathology. 1995; 26: 1154–1156
8. Sbiera S, Schmull S, Assie G, Voelker HU, Kraus L, Beyer M, Ragazzon B, Beuschlein F, Willenberg HS, Hahner S, Saeger W, Bertherat J, Allolio B, Fassnacht M. High diagnostic and prognostic value of steroidogenic factor-1 expression in adrenal tumors. Journal of Clinical Endocrinol Metab 2009; 95:161–171
9. Mondal SK, Dasgupta S, Jain P, Mandal PK, Sinha SK. Histopathological study of adrenocortical carcinoma with special reference to the Weiss system and TNM staging and the role of immunohistochemistry to differentiate it from renal cell carcinoma. J Cancer Res Ther 2013; 9:436–441
10. Sangoi AR, Fujiwara M, West RB, Montgomery KD, Bonventre JV, Higgins JP, Rouse RV, Gokden N, McKenney JK. Immunohistochemical distinction of primary adrenal cortical lesions from metastatic clear cell renal cell carcinoma: a study of 248 cases. Am J Surg Pathol 2011; 35:678–86
11. Fassnacht M, Johanssen S, Quinkler M, Bucsky P, Willenberg HS, Beuschlein F, Terzolo M, Mueller HH, Hahner S, Allolio B Limited prognostic value of the 2004 International Union Against Cancer staging classification for adrenocortical carcinoma: proposal for a Revised TNM Classification. Cancer 2009; 115:243–250

12. Lughezzani G, Sun M, Perrotte P, Jeldres C, Alasker A, Isbarn H, Budaus L, Shariat SF, Guazzoni G, Montorsi F, Karakiewicz PI The European Network for the Study of Adrenal Tumors staging system is prognostically superior to the international union against cancer-staging system: a North American validation. Eur J Cancer 2010; 46: 713–719
13. Fassnacht M, Libé R, Kroiss M, Allolio B. Adrenocortical carcinoma: a clinician's update. Nat Rev Endocrinol 2011; 7:323–335
14. Fassnacht M, Allolio B What is the best approach to an apparently nonmetastatic adrenocortical carcinoma? Clin Endocrinol 2011; 73: 561–565
15. Terzolo M, Angeli A, Fassnacht M, Daffara F, Tauchmanova L, Conton PA, Rossetto R, Buci L, Sperone P, Grossrubatscher E, Reimondo G, Bollito E, Papotti M, Saeger W, Hahner S, Koschker AC, Arvat E, Ambrosi B, Loli P, Lombardi P, Mannelli M, Bruzzi P, Mantero F, Allolio B, Dogliotti L, Berruti A Adjuvant mitotane treatment in patients with adrenocortical carcinoma. N Engl J Med 2007; 356:372–380
16. Allolio B, Fassnacht M Clinical review: adrenocortical carcinoma: clinical update. Journal of Clinical Endocrinol Metab 2006; 91: 2027–2037
17. Fassnacht M, Kroiss M, Allolio B. Update in adrenocortical carcinoma. Journal of Clinical Endocrinol Metab 2013; 98:4551–4564
18. Beuschlein F, Obracay J, Saeger W, Prognostic value of histological markers in localized adrenocortical carcinoma after complete resection. Endocr Rev. 2013; 34: 29–33
19. Sorbye H, Welin S, Langer SW, Vestermark LW, Holt N, Osterlund P, Dueland S, Hofsli E, Guren MG, Ohrling K, Birkemeyer E, Thiis-Evensen E, Biagini M, Gronbaek H, Soveri LM, Olsen IH, Federspiel B, Assmus J, Janson ET, Knigge U. Predictive and prognostic factors for treatment and survival in 305 patients with advanced gastrointestinal neuroendocrine carcinoma (WHO G3): the NORDIC NEC study. Ann Oncol. 2013; 24:152–160
20. Ronchi C, Sbiera S, Kraus L, Wortmann S, Johanssen S, Adam P, Willenberg H, Hahner S, Allolio B, Fassnacht M Expression of excision repair cross complementing group 1 and prognosis in adrenocortical carcinoma patients treated with platinum-based chemotherapy. Endocrine-Related Cancer 2009; 16 : 907–908
21. Malandrino P, Al Ghuzlan A, Castaing M, Young J, Caillou B, Travagli JP, Elias D, de Baere T, Dromain C, Paci A, Chanson P, Schlumberger M, Leboulleux S, Baudin E. Prognostic markers of survival after combined mitotane- and platinum-based chemotherapy in metastatic adrenocortical carcinoma. Endocrine-Related Cancer 2010; 17: 797–807
22. Iino K, Oki Y, Sasano H A case of adrenocortical carcinoma associated with recurrence after laparoscopic surgery. Clinical Endocrinology (Oxf) 2000; 53:243–248
23. Volante M, Terzolo M, Fassnacht M, Rapa I, Germano A, Sbiera S, Daffara F, Sperone P, Scagliotti G, Allolio B, Papotti M, Berruti A. Ribonucleotide reductase large subunit (RRM1) gene expression may predict efficacy of adjuvant mitotane in adrenocortical cancer. Clin Cancer Res. 2012; 18:3452–3461
24. Nakamura M, Miki Y, Akahira JI, Morimoto R, Satoh F, Ishidoya S, Arai Y, Suzuki T, Hayashi Y, Sasano H. An Analysis of Potential Surrogate Markers of Target-Specific Therapy in Archival Materials of Adrenocortical Carcinoma. Endocr Pathol, 2009; 20:17–23
25. Sasano H. Suzuki T. Shizawa S. Kato K. Nagura H. Transforming growth factor alpha, epidermal growth factor, and epidermal growth factor receptor expression in normal and diseased human adrenal cortex by immunohistochemistry and in situ hybridization. Modern Pathology, 1994; 7:741–746
26. Adam P, Hahner S, Hartmann M, Heinrich B, Quinkler M, Willenberg HS, Saeger W, Sbiera S, Schmull S, Voelker HU, Ströbel P, Allolio B, Fassnacht M. Epidermal growth factor receptor in adrenocortical tumors: analysis of gene sequence, protein expression and correlation with clinical outcome. Mod Pathol. 2010; 23: 1596–1604
27. Zhang J, Wang C, Gao J, Sun J, Zeng X, Wu S, Liang Z. Adrenal cortical neoplasms: a study of clinicopathological features related to epidermal growth factor receptor gene status. Diagnostic Pathology 2014; 9:19–26
28. Almeida MQ, Fragoso MC, Lotfi CF, Santos MG, Nishi MY, Costa MH, Lerario AM, Maciel CC, Mattos GE, Jorge AA, Mendonca BB, Latronico AC. Expression of insulin-like growth factor-II and its receptor in pediatric and adult adrenocortical tumors. J Clin Endocrinol Metab. 2008; 93:3524–3531
29. Fraenkel M, Gueorguiev M, Barak D, Salmon A, Grossman AB, Gross DJ. Everolimus therapy for progressive adrenocortical cancer. Endocrine. 2013; 44:187–192
30. De Martino MC, Feelders RA, de Herder WW, van Koetsveld PM, Dogan F, Janssen JA, Waaijers AM, Pivonello C, Lamberts SW, Colao A, de Krijger RR, Pivonello R, Hofland LJ. Characterization of the mTOR pathway in human normal adrenal and adrenocortical tumors. Endocr Relat Cancer 2014; 21:601–613