cases of adenocarcinoma (ADCA), 61 cases squamous cell carcinoma (SQCC) and 86 cases NET. In non-tumor tissues, we specifically observed PTPRZ1 expression in the neural cells and endocrine cells such as peripheral nerves, pancreatic islets and adrenal chromaffin cells. Representative IHC evaluations of PTPRZ1-positivity (PTPRZ1+) with anti-PTPRZ1 antibody in a variety of NETs are shown in Figure 2, for PTPRZ1-negative (PTPRZ1-) SCLC (A), PTPRZ1+ SCLC (B), MTC (C), and PanNET (D). PTPRZ1 was mainly localized in the cell membrane as well as the cytosol. We found that PTPRZ1 was detected at high frequency and intensity in a variety of human NETs including 60% of SCLCs (Figure 2E, Table 1). PTPRZ1 was expressed at much higher levels in NETs (79%) than in ADCA (9%) and SQCC (20%) (Figure 2E).

RNAi knockdown of PTPRZ1 in SCLC cell lines

To characterize further the function of PTPRZ1 in SCLC cells, we employed a genetic approach to repress *PTPRZ1* expression using by RNA interference (RNAi). For potential off-target shRNA effects, three different sequences of shRNA directed against *PTPRZ1* (sh*Z1*#1, #2 and #3) and a nontargeting shRNA (sh*LUC*) were constructed. While the introduction of the first construct sh*Z1*#1 in SCLC cells did not appear to down-regulate PTPRZ1 mRNA levels as compared to control sh*LUC* when measured by quantitative RT-PCR, significant reduction in mRNA expression of 75% using sh*Z1*#2 and 60% using sh*Z1*#3 could be observed in the expression of *PTPRZ1* in the SCLC cell lines H69 and H1930 (Figure 3A). WB analyses also revealed significant decreases in PTPRZ1 protein expression upon introduction of sh*Z1*#2 and #3, as compared to a control vector, in H69 and H1930 under normal culture conditions (Figure 3B). To measure cell surface PTPRZ1 levels in sh*Z1*-transduced SCLC cells, we used flow cytometry (FACS). FACS analysis of sh*LUC*-SCLC cells and sh*Z1*-SCLC cells also revealed significant reduction of PTPRZ1 expression on SCLC cellular surface from 29% to 6–7% in H69 cells and 37% to 9–12% in H1930 cells (Figure 3C).

Table 1 The IHC analysis of PTPRZ1 expression in human tumor tissues

Tumors	PTPRZ1−	PTPRZ1+	%
Adenocarcinoma (ADCA)			**9**
Lung ADCA	44	1	2
Breast ductal ADCA	8	2	20
Gastric ADCA	10	0	0
Pancreatic ductal ADCA	15	5	25
Colon ADCA	10	0	0
Thyroid papillary carcinoma	9	1	10
Squamous cell carcinoma (SQCC)			**20**
Lung SQCC	41	10	20
Esophagus SQCC	8	2	20
Neuroendocrine tumor (NET)			**79**
Lung NET	11	8	42
Medullary thyroid carcinoma	0	16	100
Pancreatic NET	5	26	84
Chromaffin cell tumor	0	10	100
Gastointestinal carcinoid	8	2	80

PTN induced calmodulin tyrosine phosphorylation in SCLC cells

Although our findings demonstrated that PTPRZ1 was specifically up-regulated in SCLC cells, no studies to date have suggested a functional role for PTPRZ1 in SCLC cells. As PTPRZ1 has been linked to protein tyrosine phosphatase activity, we first assessed the ability of PTPRZ1 to regulate tyrosine phosphorylation in the response to the ligand of PTPRZ1, PTN. PTN binding to the extracellular portion of PTPRZ1 brings two molecules into close proximity and consequently the phosphatase domains dimerize in a head-to-toe arrangement with the D2 domain of one molecule blocking the active site (D1) of the second molecule, leading to suppression of phosphatase activity [31,32]. To identify molecular targets regulated by PTPRZ1 in response to PTN, we assessed tyrosine-phosphorylated proteins using an anti-phosphotyrosine antibody by WB. Interestingly, we detected two specific bands that migrated just above and below 15 kDa within 30–60 min after PTN addition to SCLC cells (Figure 4A). Although it appears that those bands could be detected at low levels in the absence of PTN, the addition of PTN significantly induced phosphorylation that peaked at 1 h. Since calmodulins (CaM) are highly abundant, 17 kDa proteins in the mammalian brain, nervous and endocrine systems and directly interact with the intracellular domain of PTPR members [33,34], we hypothesized that PTPRZ1 may normally dephosphorylate the phosphorylated tyrosine residue at Tyr99 of CaM. To test this idea, we assessed the phosphorylation of CaM using an anti- phospho-Tyr99-CaM (p-CaM) Ab and determined that the upper band could indeed be identified as CaM (Figure 4B).

PTPRZ1 is required for the tyrosine phosphorylation of CaM induced by PTN

To verify that the addition of PTN facilitated CaM phosphorylation specifically through its receptor PTPRZ1, we utilized the H69 and H1930 cell lines in which sh*Z1* was used to knock down PTPRZ1 expression. Although PTN induced tyrosine phosphorylation of CaM in H69 cells that expressed the control sh*LUC*, the ablation of

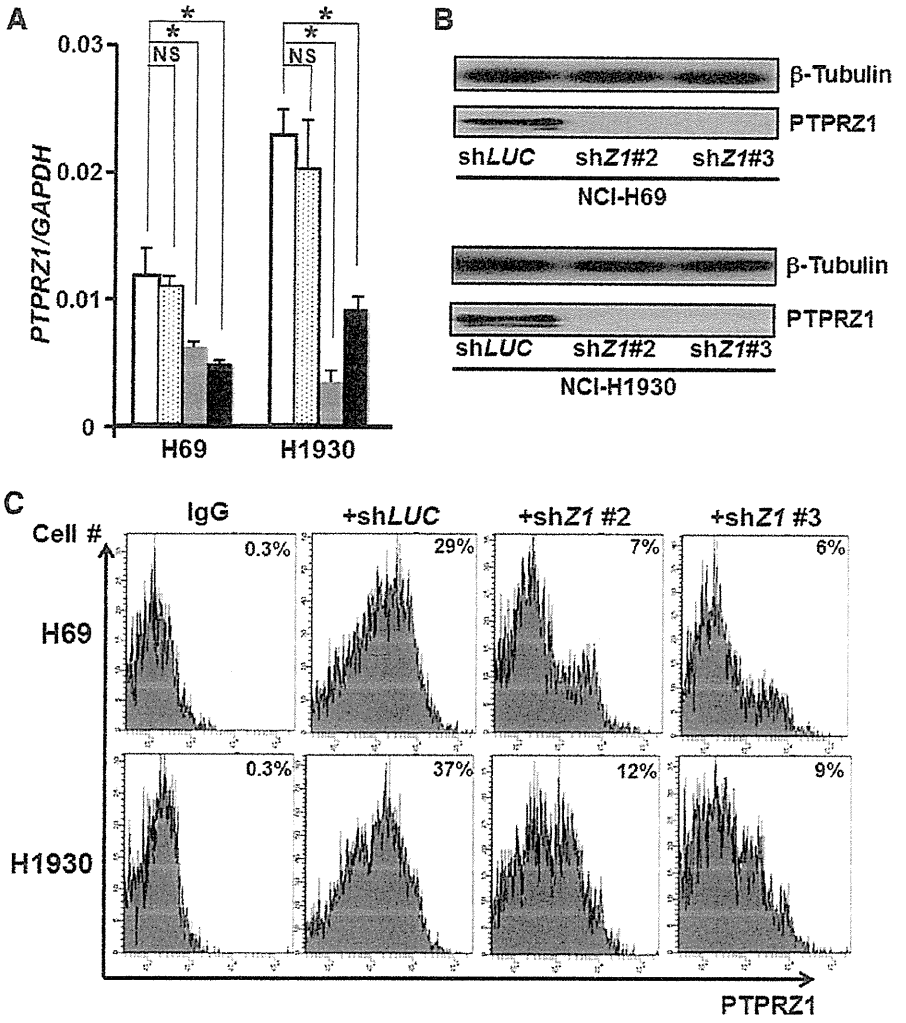

Figure 3 PTPRZ1 expression was downregulated by shRNA in SCLC cell lines. A, shRNA targeting *PTPRZ1* (sh*Z1*) successfully knock-downed *PTPRZ1* mRNA by up to 75% in the SCLC cell lines, H69 and H1930, as compared to negative control construct expressing sh*LUC*. White bars = sh*LUC*, dotted bars = sh*Z1*#1, gray bars = sh*Z1*#2, black bars = sh*Z1*#3. Error bars represent SD. Asterisk denotes $P < 0.05$ using Student's *t* test, while NS denotes non-significant change. **B**, PTPRZ1 downregulation in H69 and H1930 was confirmed by Western blot, using β-tubulin as control for protein levels. **C**, FACS analysis of surface PTPRZ1 protein expression on H69 and H1930 cells, with sh*Z1* down-regulating PTPRZ1 expression levels.

PTPRZ impaired PTN-induced CaM tyrosine phosphorylation in cells that expressed either of the two sh*Z1* constructs (Figure 4C). We confirm that PTPRZ1 was indispensable for the PTN-induced p-CaM in H1930 cells (Figure 4D).

As the serum levels of PTN were elevated in most SCLC patients in comparison to healthy controls [12], we thought PTPRZ1 expression might be correlate with the expression of phosphorylated CaM. To assess whether PTPRZ1-CaM regulation also occurred *in vivo* in human tissue, we stained for CaM and p-CaM. Indeed we found that the expression of PTPRZ1 and p-CaM was statistically correlated in human lung NET tissues (Figure 4E). These data thus demonstrate that ablation of PTPRZ1 prevents PTN-stimulated tyrosine phosphorylation of CaM in PTN-stimulated SCLC cells; the data indicate that endogenous PTPRZ1 is required for PTN-stimulating tyrosine phosphorylation of CaM in SCLC cells.

PTPRZ1 regulates tumor progression of SCLC in xenograft model

Many PTPRs play an important role as tumor suppressors [9], yet PTPRZ1 has a role in cell migration and tumor growth *in vivo* in glioma studies [20]. To determine whether overexpressed PTPRZ1 acts as a tumor suppressor or tumor promoter in human NETs, we used the severe combined immunodeficiency (*SCID*) murine

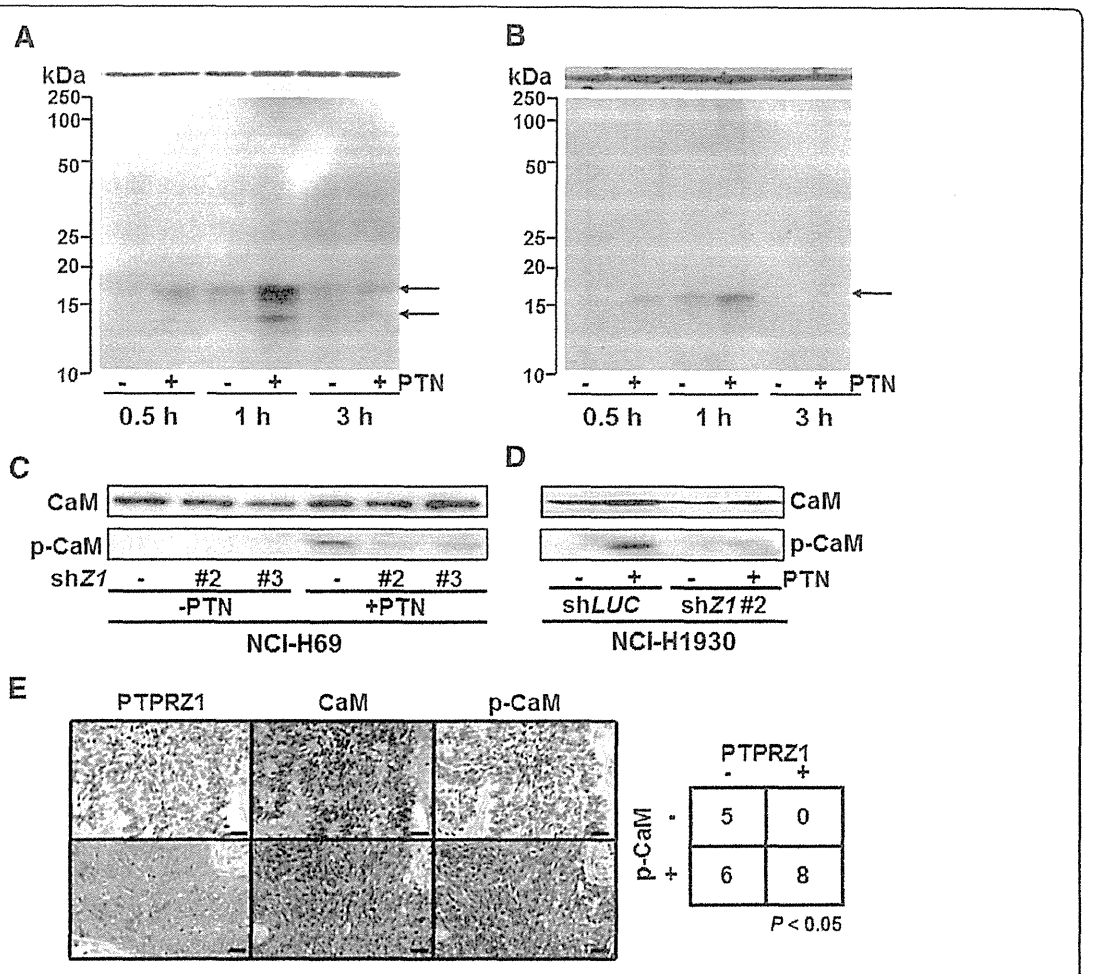

Figure 4 PTPRZ1 regulates tyrosine phosphorylation in SCLC cells. A, Tyrosine phosphorylated proteins in H69 cells were detected by Western blot with anti-pTyr antibody (4 G10) with β-catenin as loading control on top. **B**, Tyrosine phosphorylated CaM was detected by Western blot with anti-phosphorylated CaM, with total CaM on top as loading control in H69 cells. **C** and **D**, sh*Z1*s block PTN-stimulated tyrosine phosphorylation of CaM in H69 (**C**) and H1930 (**D**). PTPRZ1 promotes the phosphorylation of calmodulin in response to PTN. **E**, Correlated expression of PTPRZ1 and tyrosine phosphphorylated CaM (pCaM) in human lung NET tissues. Representative negative (–) or positive (+) images were shown. Scale bars were 20 μm (Magnification X40). $P < 0.05$ was considered statistically significant.

xenograft model subcutaneously transplanted with human SCLC cells. 2 x 10^6 H69 cells expressing either sh*LUC* (H69 + sh*LUC*) as a control or sh*Z1*#2 (H69 + sh*Z1*#2) were subcutaneously transplanted into the flanks of *SCID* mice (n = 7) and tumor size was measured over time. In this mouse model, H69 + sh*LUC* cells started to grow exponentially at 7 days post-transplant and progressively form tumor masses for 5 weeks (Figure 5A). In contrast, the H69 + sh*Z1*#2 cells were impaired for tumor formation until 3 weeks post-transplant such that tumors were barely recognized under the skin and were about 3-fold smaller than those in H69 + sh*LUC* cells (Figure 5A and B). Gross examination of H69 + sh*Z1*#2 tumors revealed a dramatic loss of SCLC pathology in the tumor (Figure 5B). To exclude the possibility of off-target effects of shZ2#2, we subcutaneously transplanted H69 cells expressing either sh*LUC* (H69 + sh*LUC*) as a control or sh*Z1*#3 (H69 + sh*Z1*#3) into the flanks of *SCID* mice (n = 7) and we obtained similar results (Figure 5C). In another SCLC cell line, H1930, the reduction of PTPRZ1 expression decreased the rate of tumor formation under the skin in *SCID* mice as compared to the cells expressing the sh*LUC* control (Figure 5D). These results provide proof that PTPRZ1 regulates tumor growth *in vivo* and has an oncogenic function in NET progression.

Discussion

Here we demonstrate that PTPRZ1 specifically exists in human NET tissues and PTPRZ1 has an important oncogenic role in the tumor progression of SCLC in the murine xenograft model. We also found that PTPRZ1

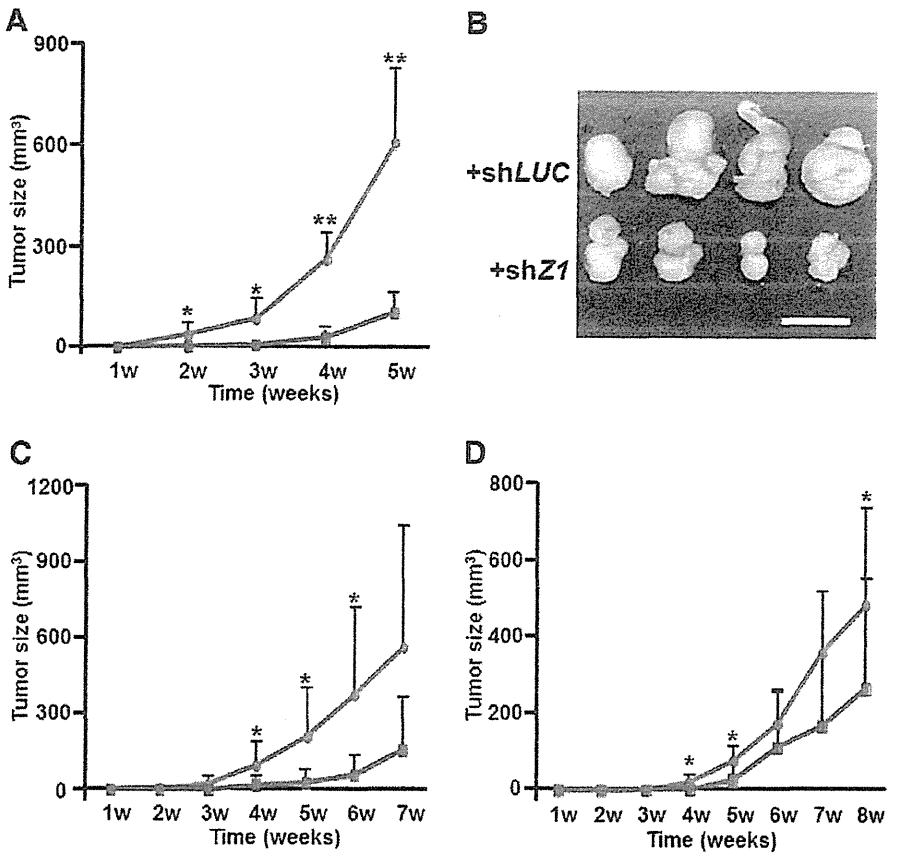

Figure 5 PTPRZ1 regulates tumor progression in a SCLC xenograft mouse model. A and **B**, *in vivo* growth of SCLC NCI-H69 expressing sh*LUC* (blue diamonds) and sh*Z1*#2 (red squares) in *SCID* mice. The loss of *PTPRZ1* in cells expressing shZ1#2 inhibited tumor growth as compared to cells expressing the control sh*LUC*. Tumor size (mm^3) over period (**A**) and gross tumor pathology 5 weeks post-transplant are shown (**B**). Scale bar indicates 10 mm. **C** and **D**, Deficient *in vivo* growth of tumors was confirmed using H69 cells expressing sh*Z1*#3 (**C**) and H1930 cells expressing sh*Z1*#2. Control SCLC cells with shLUC are shown as blue diamonds while cells with shZ1 are shown as red squares. Error bars are SD. Asterisk, $P < 0.05$; double asterisk, $P < 0.01$; both using Student's *t* test.

regulates the tyrosine phosphorylation of CaM in the response to PTN in SCLC cells. Our results indicate that the putative tumor suppressor family PTPR can support tumor progression and is required for the tyrosine phosphorylation of CaM. This study supports the idea that a new signaling pathway involving PTPRZ1 could be a feasible target for treatment of cancers. The combination of our cellular and xenograft model findings advocates for the future preclinical testing of antibody therapy or small molecule inhibitors of PTPRZ1 for the treatment of NETs and SCLC.

The linkage between oncogenic PTPRZ1 function and CaM phosphorylation is still unclear. Perez-Pinera and colleagues demonstrated that phosphorylation of Anaplastic lymphoma kinase (ALK) in PTN-stimulated cells is mediated through the PTN/PTPRZ1 signaling pathway [35], indicating that ALK might phosphorylate CaM. Further experiments are needed to address the possibility of PTN mediating its effects via ALK [35] in SCLC cells, the effects of PTN deletion on tumor growth, and the mechanism of PTN/PTPRZ1 autocrine regulation in NET cells. CaM can bind up to four calcium ions, and can undergo post-translational modifications such as phosphorylation, acetylation, methylation and proteolytic cleavage, each of which can potentially modulate its actions [34]. A prior biochemical study showed that tyrosine phosphorylation increased the association of CaM with nitric oxide synthase (NOS) [36]. Because nitric oxide (NO) and NOS are ubiquitous in malignant tumors and known to exert pro-tumor effects [37,38], PTPRZ1 may regulate NO production in SCLC cells by changing the tyrosine phosphorylation status of CaM. Tumor cell-derived NO promotes tumor progression by induction of tumor-cell invasion, proliferation and the expression of angiogenic factors [37,38]. Indeed a recent research article demonstrated that glioma stem cell proliferation and tumor growth are promoted by iNOS [39].

With regards to another aspect of its oncogenic role, PTPRZ1 has a huge extracellular domain consisting of a alpha-carbonic anhydrase domain (CA), chondroitin sulfate proteoglycans (CS-PGs), and a fibronectin type-III domain (FNIII). PTPRZ1 expression is dramatically induced by hypoxic stress through HIF-2α [19], suggesting that PTPRZ1 may have an important role under hypoxic conditions. Recently, Jeong's research group reported that CA was dramatically up-regulated in human SCLC tissues by proteomic analysis [40]. A possible speculation is that the CA domain of PTPRZ1 could have an important function for tumor progression of SCLC and further studies will be required to address this issue.

Conclusions

We found that PTPRZ1 has an important oncogenic role in tumor progression in the murine xenograft model of SCLCs. Moreover we demonstrate that the binding of PTPRZ1 to its ligand PTN inactivates phosphatase activity, resulting in tyrosine phosphorylation of CaM in human tumors. These results indicate that a new signaling pathway involving PTPRZ1 could be a feasible target for treatment of NETs.

Abbreviations

SCLC: Small cell lung carcinoma; PTP: Protein tyrosine phosphatase; PTPRZ1: Protein tyrosine phosphatase receptor Z1; NETs: Neuroendocrine tumors; PTN: Pleiotrophin; CaM: Calmodulin; shRNA: Small Hairpin RNA.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Histological diagnostics for pathological human tissues were carried out by GI, MK, SF, TK and AO. AO conceived the study. All experiments were optimized and performed by HM. Manuscript were written by HM and revised by YH, GI and AO. All authors have read and approved this manuscript.

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References

1. Chen H, Sippel RS, O'Dorisio MS, Vinik AI, Lloyd RV, Pacak K: The North American Neuroendocrine Tumor Society consensus guideline for the diagnosis and management of neuroendocrine tumors: pheochromocytoma, paraganglioma, and medullary thyroid cancer. *Pancreas* 2010, 39(6):775–783.
2. Klimstra DS, Modlin IR, Coppola D, Lloyd RV, Suster S: The pathologic classification of neuroendocrine tumors: a review of nomenclature, grading, and staging systems. *Pancreas* 2010, 39(6):707–712.
3. Phan AT, Oberg K, Choi J, Harrison LH Jr, Hassan MM, Strosberg JR, Krenning EP, Kocha W, Woltering EA, Maples WJ: NANETS consensus guideline for the diagnosis and management of neuroendocrine tumors: well-differentiated neuroendocrine tumors of the thorax (includes lung and thymus). *Pancreas* 2010, 39(6):784–798.
4. Kulke MH, Anthony LB, Bushnell DL, de Herder WW, Goldsmith SJ, Klimstra DS, Marx SJ, Pasieka JL, Pommier RF, Yao JC, *et al*: NANETS treatment guidelines: well-differentiated neuroendocrine tumors of the stomach and pancreas. *Pancreas* 2010, 39(6):735–752.
5. William WN Jr, Glisson BS: Novel strategies for the treatment of small-cell lung carcinoma. *Nat Rev Clin Oncol* 2011, 8(10):611–619.
6. Kim YH, Mishima M: Second-line chemotherapy for small-cell lung cancer (SCLC). *Cancer Treat Rev* 2011, 37(2):143–150.
7. Stoker AW: Protein tyrosine phosphatases and signalling. *J Endocrinol* 2005, 185(1):19–33.
8. Tonks NK: Protein tyrosine phosphatases: from genes, to function, to disease. *Nat Rev Mol Cell Biol* 2006, 7(11):833–846.
9. Julien SG, Dube N, Hardy S, Tremblay ML: Inside the human cancer tyrosine phosphatome. *Nat Rev Cancer* 2011, 11(1):35–49.
10. Wang Z, Shen D, Parsons DW, Bardelli A, Sager J, Szabo S, Ptak J, Silliman N, Peters BA, van der Heijden MS, *et al*: Mutational analysis of the tyrosine phosphatome in colorectal cancers. *Science* 2004, 304(5674):1164–1166.
11. Shitara K, Yamada H, Watanabe K, Shimonaka M, Yamaguchi Y: Brain-specific receptor-type protein-tyrosine phosphatase RPTP beta is a chondroitin sulfate proteoglycan *in vivo*. *J Biol Chem* 1994, 269(31):20189–20193.
12. Jager R, List B, Knabbe C, Souttou B, Raulais D, Zeiler T, Wellstein A, Aigner A, Neubauer A, Zugmaier G: Serum levels of the angiogenic factor pleiotrophin in relation to disease stage in lung cancer patients. *Br J Cancer* 2002, 86(6):858–863.
13. Kadomatsu K, Muramatsu T: Midkine and pleiotrophin in neural development and cancer. *Cancer Lett* 2004, 204(2):127–143.
14. Liu YT, Shang D, Akatsuka S, Ohara H, Dutta KK, Mizushima K, Naito Y, Yoshikawa T, Izumiya M, Abe K, *et al*: Chronic oxidative stress causes amplification and overexpression of ptprz1 protein tyrosine phosphatase to activate beta-catenin pathway. *Am J Pathol* 2007, 171(6):1978–1988.
15. Tamura H, Fukada M, Fujikawa A, Noda M: Protein tyrosine phosphatase receptor type Z is involved in hippocampus-dependent memory formation through dephosphorylation at Y1105 on p190 RhoGAP. *Neurosci Lett* 2006, 399(1–2):33–38.
16. Pariser H, Perez-Pinera P, Ezquerra L, Herradon G, Deuel TF: Pleiotrophin stimulates tyrosine phosphorylation of beta-adducin through inactivation of the transmembrane receptor protein tyrosine phosphatase beta/zeta. *Biochem Biophys Res Commun* 2005, 335(1):232–239.
17. Pariser H, Ezquerra L, Herradon G, Perez-Pinera P, Deuel TF: Fyn is a downstream target of the pleiotrophin/receptor protein tyrosine phosphatase beta/zeta-signaling pathway: regulation of tyrosine phosphorylation of Fyn by pleiotrophin. *Biochem Biophys Res Commun* 2005, 332(3):664–669.
18. Meng K, Rodriguez-Pena A, Dimitrov T, Chen W, Yamin M, Noda M, Deuel TF: Pleiotrophin signals increased tyrosine phosphorylation of beta beta-catenin through inactivation of the intrinsic catalytic activity of the receptor-type protein tyrosine phosphatase beta/zeta. *Proc Natl Acad Sci U S A* 2000, 97(6):2603–2608.
19. Wang V, Davis DA, Veeranna RP, Haque M, Yarchoan R: Characterization of the activation of protein tyrosine phosphatase, receptor-type, Z polypeptide 1 (PTPRZ1) by hypoxia inducible factor-2 alpha. *PLoS One* 2010, 5(3):e9641.
20. Ulbricht U, Eckerich C, Fillbrandt R, Westphal M, Lamszus K: RNA interference targeting protein tyrosine phosphatase zeta/receptor-type

protein tyrosine phosphatase beta suppresses glioblastoma growth in vitro and *in vivo*. *J Neurochem* 2006, 98(5):1497–1506.
21. Foehr ED, Lorente G, Kuo J, Ram R, Nikolich K, Urfer R: **Targeting of the receptor protein tyrosine phosphatase beta with a monoclonal antibody delays tumor growth in a glioblastoma model.** *Cancer Res* 2006, 66(4):2271–2278.
22. Ulbricht U, Brockmann MA, Aigner A, Eckerich C, Muller S, Fillbrandt R, Westphal M, Lamszus K: **Expression and function of the receptor protein tyrosine phosphatase zeta and its ligand pleiotrophin in human astrocytomas.** *J Neuropathol Exp Neurol* 2003, 62(12):1265–1275.
23. Muller S, Kunkel P, Lamszus K, Ulbricht U, Lorente GA, Nelson AM, von Schack D, Chin DJ, Lohr SC, Westphal M, *et al*: **A role for receptor tyrosine phosphatase zeta in glioma cell migration.** *Oncogene* 2003, 22(43):6661–6668.
24. Feng ZJ, Gao SB, Wu Y, Xu XF, Hua X, Jin GH: **Lung cancer cell migration is regulated via repressing growth factor PTN/RPTP beta/zeta signaling by menin.** *Oncogene* 2010, 29(39):5416–5426.
25. Simms E, Gazdar AF, Abrams PG, Minna JD: **Growth of human small cell (oat cell) carcinoma of the lung in serum-free growth factor-supplemented medium.** *Cancer Res* 1980, 40(12):4356–4363.
26. Carney DN, Gazdar AF, Bepler G, Guccion JG, Marangos PJ, Moody TW, Zweig MH, Minna JD: **Establishment and identification of small cell lung cancer cell lines having classic and variant features.** *Cancer Res* 1985, 45(6):2913–2923.
27. Nitadori J, Ishii G, Tsuta K, Yokose T, Murata Y, Kodama T, Nagai K, Kato H, Ochiai A: **Immunohistochemical differential diagnosis between large cell neuroendocrine carcinoma and small cell carcinoma by tissue microarray analysis with a large antibody panel.** *Am J Clin Pathol* 2006, 125(5):682–692.
28. Ma Y, Ye F, Xie X, Zhou C, Lu W: **Significance of PTPRZ1 and CIN85 expression in cervical carcinoma.** *Arch Gynecol Obstet* 2011, 284(3):699–704.
29. Makinoshima H, Dezawa M: **Pancreatic cancer cells activate CCL5 expression in mesenchymal stromal cells through the insulin-like growth factor-I pathway.** *FEBS Lett* 2009, 583(22):3697–3703.
30. Chow JP, Fujikawa A, Shimizu H, Suzuki R, Noda M: **Metalloproteinase- and gamma-secretase-mediated cleavage of protein-tyrosine phosphatase receptor type Z.** *J Biol Chem* 2008, 283(45):30879–30889.
31. Barr AJ, Ugochukwu E, Lee WH, King ON, Filippakopoulos P, Alfano I, Savitsky P, Burgess-Brown NA, Muller S, Knapp S: **Large-scale structural analysis of the classical human protein tyrosine phosphatome.** *Cell* 2009, 136(2):352–363.
32. Blanchetot C, Tertoolen LG, Overvoorde J, den Hertog J: **Intra- and intermolecular interactions between intracellular domains of receptor protein-tyrosine phosphatases.** *J Biol Chem* 2002, 277(49):47263–47269.
33. Liang L, Lim KL, Seow KT, Ng CH, Pallen CJ: **Calmodulin binds to and inhibits the activity of the membrane distal catalytic domain of receptor protein-tyrosine phosphatase alpha.** *J Biol Chem* 2000, 275(39):30075–30081.
34. Benaim G, Villalobo A: **Phosphorylation of calmodulin. Functional implications.** *Eur J Biochem* 2002, 269(15):3619–3631.
35. Perez-Pinera P, Zhang W, Chang Y, Vega JA, Deuel TF: **Anaplastic lymphoma kinase is activated through the pleiotrophin/receptor protein-tyrosine phosphatase beta/zeta signaling pathway: an alternative mechanism of receptor tyrosine kinase activation.** *J Biol Chem* 2007, 282(39):28683–28690.
36. Corti C, Leclerc L'Hostis E, Quadroni M, Schmid H, Durussel I, Cox J, Dainese Hatt P, James P, Carafoli E: **Tyrosine phosphorylation modulates the interaction of calmodulin with its target proteins.** *Eur J Biochem* 1999, 262(3):790–802.
37. Williams EL, Djamgoz MB: **Nitric oxide and metastatic cell behaviour.** *Bioessays* 2005, 27(12):1228–1238.
38. Fukumura D, Kashiwagi S, Jain RK: **The role of nitric oxide in tumour progression.** *Nat Rev Cancer* 2006, 6(7):521–534.
39. Eyler CE, Wu Q, Yan K, Macswords JM, Chandler-Militello D, Misuraca KL, Lathia JD, Forrester MT, Lee J, Stamler JS, *et al*: **Glioma stem cell proliferation and tumor growth are promoted by nitric oxide synthase-2.** *Cell* 2011, 146(1):53–66.
40. Jeong HC, Kim GI, Cho SH, Lee KH, Ko JJ, Yang JH, Chung KH: **Proteomic analysis of human small cell lung cancer tissues: up-regulation of coactosin-like protein-1.** *J Proteome Res* 2011, 10(1):269–276.

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Elastic laminal invasion in colon cancer: diagnostic utility and histological features

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Primary tumors of the colorectal cancers are assessed pathologically based on the tumor spread into the bowel wall. The assessment of serosal involvement, which may be relevant to pT4, can be challenging for pathologists, making the consistency of diagnoses questionable. As solutions to this problem, the following two strategies could be adopted. One would be to use special staining or immunohistochemical staining techniques for diagnostic assistance. The other would be to construct recommendations for the assessment of tumor spreading and to obtain a world-wide consensus on the criteria used to assess tumor spreading. Using elastic staining, we previously reported that peritoneal elastic laminal invasion (ELI) could be objectively determined and would likely contribute to a simplified and more objective stratification of deep tumor invasion around the peritoneal surface. We also noted the importance of sampling, staining, and histo-anatomical knowledge in the application of elastic staining during routine pathological diagnosis. Here we review the history of primary tumor stratification leading to the present TNM classification and report on the current status of pathological assessments made at our hospital to summarize what has been established and what is further required for the pathological diagnosis of tumor spreading in patients with colorectal cancer.

Keywords: colon cancer, pathology, diagnosis, elastic lamina, tumor spread

INTRODUCTION

Since the first categorization efforts reported by Lockhart-Mummery (1926–1927), primary colorectal cancers have been consistently stratified based on the extent of their spreading into the bowel wall (Dukes, 1932; Jass et al., 1987; Newland et al., 1995). Deep tumor invasion around the peritoneal surface has also been reported as a prognostic factor (invasion through all the layers, peritoneal involvement, or direct spreading involving a free serosal surface; Astler and Coller, 1954; Shepherd et al., 1997). These reports were base for the 7th TNM classification (Sobin et al., 2009). Reviewing these reports should help to renew our understanding of what has been established and what is further required for the pathological diagnosis of tumor spreading. Using elastica staining, we previously showed that peritoneal elastic laminal invasion (ELI) could be objectively determined and would likely contribute to a simplified and more objective stratification of deep tumor invasion around the peritoneal surface (Kojima et al., 2010). We also note the importance of sampling, staining, and histo-anatomical knowledge to apply elastica staining to routine pathological diagnosis. In this review, we will reflect on the brilliant achievements in the assessment of tumor spreading in colorectal cancer. We will also report on the current status of pathological assessments made at our hospital and will expose associated problems that will require solutions in the future. In addition to the diagnostic criteria, we also feel that a comprehensive minimum consensus is required for pathology protocols, including sampling and staining protocols in the future. Next, in addition to their prognostic relevance, areas with ELI exhibit marked fibrosis and tumor budding. These findings suggest that ELI areas may actively induce metastasis and such observations may lead to future biological investigations. On the other hand, ELI can only be a surrogate marker of deep tumor invasion. Therefore, we reviewed our records and showed that ELI was a superior prognostic marker compared with the depth of tumor invasion, suggesting that the ELI tumor area may play an active role in the metastasis of colorectal cancer. Finally, we summarize several biological topics that may be relevant to our pathological findings.

HISTORY OF THE ASSESSMENT OF TUMOR SPREADING IN COLORECTAL CANCER

Many early reports of the classification of tumor spreading focused mainly on rectal cancer. In the first report by Lochart-Mummery, tumor spreading and metastasis was classified as follows: (A) favorable cases, tumor did not invade the muscularis; no nodes involved; (B) medium cases, tumor invaded muscular coat; no extensive involvement of nodes; and (C) very bad cases, tumor large, and fixed; or extensive involvement of nodes. In the classical Duke's classification, tumor spreading was classified according to the presence of extra-rectal tissue. And in the modified Duke's classification published in 1958, tumor spreading was classified as (1) confined to the bowel wall, (2) commencing to invade the extra-rectal tissues, (3) well established in the mesentery, or (4)

deeply invasive, possibly into neighboring organs. Also, in the modified Duke's classification reported by Kirklin et al. (1949) divided as follows: type A, lesion limited to the mucosa; Type B1, lesion extended into the muscularis propria, but not penetrating it; Type B2, lesion penetrated through the muscularis propria. They completed the construction of an outline for the current pT1-3 stages in the current TNM classification. They also found that the extent of local spreading was associated with a poor 5 year survival rate, and their report was followed by studies measuring the depth of local spreading in rectal cancer (Cawthorn et al., 1990; Shirouzu et al., 2011). As reported by Kirklin et al. we now know that most of these classifications can also be used for the assessment of colon cancer. On the other hand, some histo-anatomical differences exists, the largest is the existence of the peritoneal coat, leading to differences in the pathological criteria for pT4 colon cancer, compared with those used for rectal cancer. Free mesothelial surface involvement or local peritoneal involvement (lPI), which relevant to present pT4 was reported by Newland et al. (1993) and Shepherd et al., 1995; Ludeman and Shepherd, 2005). They challenged to sub-stage tumor spreading beyond the bowel wall using the peritoneal surface. Using the modified Australian ClinicoPathological Staging System (ACPS), Newland et al. showed that tumor spreading involving a free mesothelial surface was a prognostic factor. Shepherd et al. showed that LPI Group 3 and 4 were predictors of a poor prognosis in patients with colorectal cancer. Apart from the details of these definitions, they found colorectal cancer spreading just around or over the outer surface of the bowel wall was associated with a poor prognosis, and many data support their criteria (we termed "tumor involvement of free mesothelial surface," "pT4," and "LPI Group 3 and 4" as serosal involvement in following context). Using a fully standardized pathology, other strategies for the treatment of colorectal cancer may be developed (Wolpin and Mayer, 2008). For example, adjuvant chemotherapy may provide a benefit to colonic cancer patients with serosal involvement who do not have lymph node metastasis (stage IIB; Morris et al., 2007).

CURRENT STATUS OF MACROSCOPIC AND HISTOLOGICAL EXAMINATION TO ASSESS SEROSAL INVOLVEMENT AND EXPOSURE OF DIAGNOSTIC PROBLEMS

Detailed macroscopic observation and sampling are essential steps in making an accurate and objective diagnosis. Macroscopic features from luminal and serosal side were shown in **Figures 1A,C**, respectively. The slices we made are shown in black line in **Figures 1B,D**. In our department, surgically resected specimens are extended using a pin and cork board. After 24 h of fixation in 10% buffered formalin, macroscopic observations of the luminal, and serosal sides are performed, and the deepest area of the ulcer floor and indentations of the serosal surface are identified. In the tenial area of the colonic wall, serosal indentation is easy to identify (**Figure 1D**, arrow). On the other hand, serosal involvement on the adipose rich mesenteric side is often difficult to identify (**Figure 1D**, arrow head). In the normal state, fatty appendices of the colon form peritoneum-lined crevice. Serosal involvement, especially free-floating tumor cells are often found in this area. Macroscopically, the existence of serosal involvement in this area can be speculated based on widening or brownish change in the crevice near the deepest area of ulcer floor (**Figure 1D**, arrow head). Therefore, we make the first cross-sectioning of the tumor in a manner that places the cut through these above-mentioned areas (**Figures 1B,D**). A parallel slice is then made after the first slice. Indentations of the serosal surface should be identified on the cut surface and sampled extensively (**Figure 1E**, arrow and arrow head). In addition, case with serosal involvement frequently shows macroscopic stricture (Miyamoto et al., 2001). Serosal involvement can also be observed as a macroscopic streak sign on the cut surface, providing a useful clue for identification (Inomata et al., 1998; **Figure 1E**, arrow head).

Histologically, many pathologists report tumor spreading according to the definition in TNM classification. However, a recent study has questioned the reproducibility of serosal involvement. Similarly, the diagnosis of pT3 or pT4 is often difficult. This situation has been well described by Stewart et al. (2007b, 2011). They pointed out the histo-anatomical and histopathological characteristics of peritoneal tissue. Histo-anatomically, peritoneal tissue consists of the mesothelium and submesothelial layer, which coats the colonic wall and the surrounding pericolic adipose tissue (Mills, 2007). Therefore, especially around the adipose tissue, the peritoneal surface is not smooth, but instead exhibits peritoneal clefts or peritoneal reflection where serosal involvement is frequently seen (**Figure 1C**).

Difference in the level of H.E slides and the difference of the elastic staining used can influence on the diagnosis of both serosal invasion and ELI. In our hospital, histological evaluation is performed by two levels of H.E staining and one routine Elastica staining using Maeda Resorcin-Fuchshin Solution as described previously (Kojima et al., 2011). Histological slides of the same tumor section with **Figure 1E** was shown in **Figures 2A–F**. Arrow and arrow head in **Figure 1E** are concordant with that in **Figures 2A,B**. High power magnification of arrow is corresponded to **Figures 2C,D**. And that of arrow head is corresponded to **Figures 2E,F**. We want to stress that histological deepest area of tumor invasion is concordant with macroscopic indentation. Furthermore, peritoneal elastic lamina is retracting toward the tumor (**Figures 2B,D,F**). Histopathologically, although the normal submesothelial layer contains few cells, this tissue frequently shows fibro inflammatory changes when tumor cells invade areas near this tissue (**Figure 2C**). In colorectal cancer, fibroinflammation is often seen near the invasive front, but this phenomenon is much more prominent in the tumor area with ELI (**Figure 2C**). When the fibrosis is prominent, a peritoneal surface elevation toward the tumor is often seen (**Figure 2A**, arrow). In some cases, prominent fibrosis with a non-cellular matrix component forms a fibrotic focus (Nishimura et al., 1998; **Figure 2G**). This characteristic morphological features also often found in the tumor area with ELI (**Figure 2H**). Using low-power observations, a scar-like radiating fibrosclerotic core is observed (Van den Eynden et al., 2007; **Figures 3A,B**). Using high power magnification, the fibroblasts arranged in a storiform pattern (**Figures 3C,D**). Marked collagenization is also found. The tumor cell clusters become sparser within area of fibrosis, and budding foci are often seen within this area (**Figure 3D**).

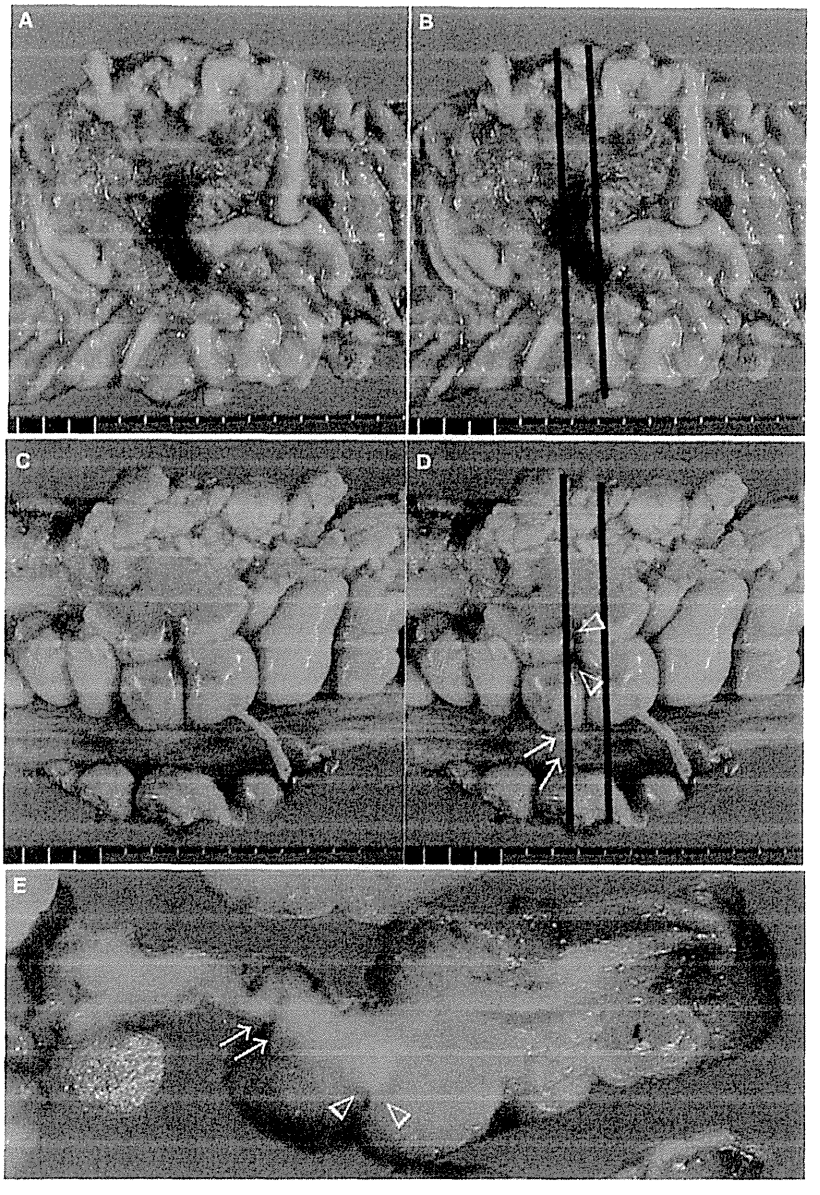

FIGURE 1 | Macroscopic assessment of serosal invasion in colonic cancer. Macroscopic features from luminal side **(A,B)**, serosal side **(C,D)**, and cut surface of the lesion **(E)**. In this case, we identified both a serosal indentation (arrow) and the widening and brownish change of the crevice (arrow head) in **(D)**. The first two slices through these lesions are shown in **(B,D)**, and the cut surface is shown in **(E)**. On the cut surface, the indentation of the serosal surface (arrow) and a streak sign (arrow head) were identified. These areas were sampled for histological examination in **Figure 2**.

Inflammatory cells, including macrophages, are often seen around these lesions. CD68-positive or CD204-positive macrophages are found predominantly around the peritoneal elastic lamina and fibrotic focus (**Figures 3E,F**). Such a variety of histopathological alterations makes it difficult to determine serosal involvement by H.E stain alone. Accordingly, even with optimized sampling, the frequency of serosal invasion varies in reports, and the diagnostic concordance is relatively low (Compton, 2003). Therefore, consistent data regarding tumor spreading as assessed at different hospitals is impossible, and this fact hinders the design of multicenter therapeutic trials for high risk stage II colon cancer patients. To overcome this situation, two approaches can be considered. One approach is to establish more detailed diagnostic criteria. Recommendations including assessment and sample preparation protocols may also be useful. In fact, extensive sampling using detailed macroscopic observations definitely improves the accuracy of diagnoses of serosal involvement (Ludeman and Shepherd, 2005). The other approach is to use special staining or immunohistochemical staining techniques to provide diagnostic assistance.

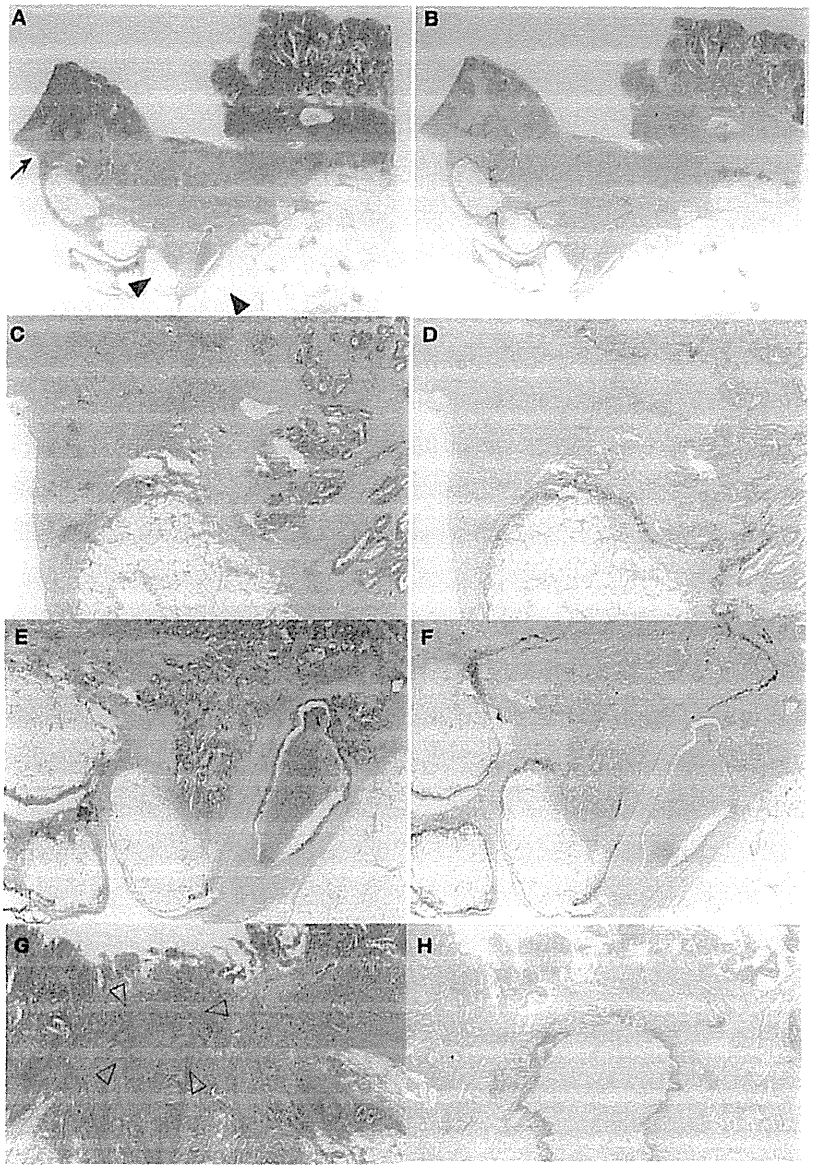

FIGURE 2 | Histological features of serosal invasion in the case shown in Figure 1. The arrows and arrow heads in the macroscopic picture shown in **Figure 1E** are identical to the arrows and arrow heads in the histologic picture shown in **(A,B)**. Elastica staining picture from the serial section was obtained, and H.E stained **(A,C,E,G)** is same area with elastic stained **(B,D,F,H)**, respectively. **(A–F)** Histological features showing peritoneal surface elevation toward the tumor (arrow) and a streak sign (arrow head) in a colorectal cancer specimen. The tumor had deeply invaded the region near the serosal surface, and prominent fibroinflammation was visible in the area, identical to the macroscopic findings **(C,E)**. ELI is also seen in the area with macroscopic findings **(D,F)**. In some cases, prominent fibrosis with a non-cellular matrix component forms a fibrotic focus **(G)**. Using elastica staining we detect fibrotic focus just beneath the peritoneal elastic lamina **(H)**.

WAYS TO CONSTRUCT STANDARDIZED ASSESSMENTS OF SEROSAL INVOLVEMENT TO OVERCOME CURRENT DIAGNOSTIC PROBLEMS

ESTABLISHMENT OF STANDARDIZED CRITERIA

Pathologists must regularly make difficult choices to diagnose serosal invasion. Diagnostic recommendations or criteria may lessen such difficulties and may even improve the concordance of pathological diagnoses. Any group may decide to establish criteria (informal approach). On the other hand, a criteria established based on more structural surveys would be acceptable for many pathologists, and are increasingly being used to develop clinical guidelines. Klimstra et al. (2010) used this structural survey of "Delphi method" to establish a consensus regarding the reporting of neuroendocrine tumors. Such a method may also contribute to establishing consensus-based criteria for pathological serosal invasion.

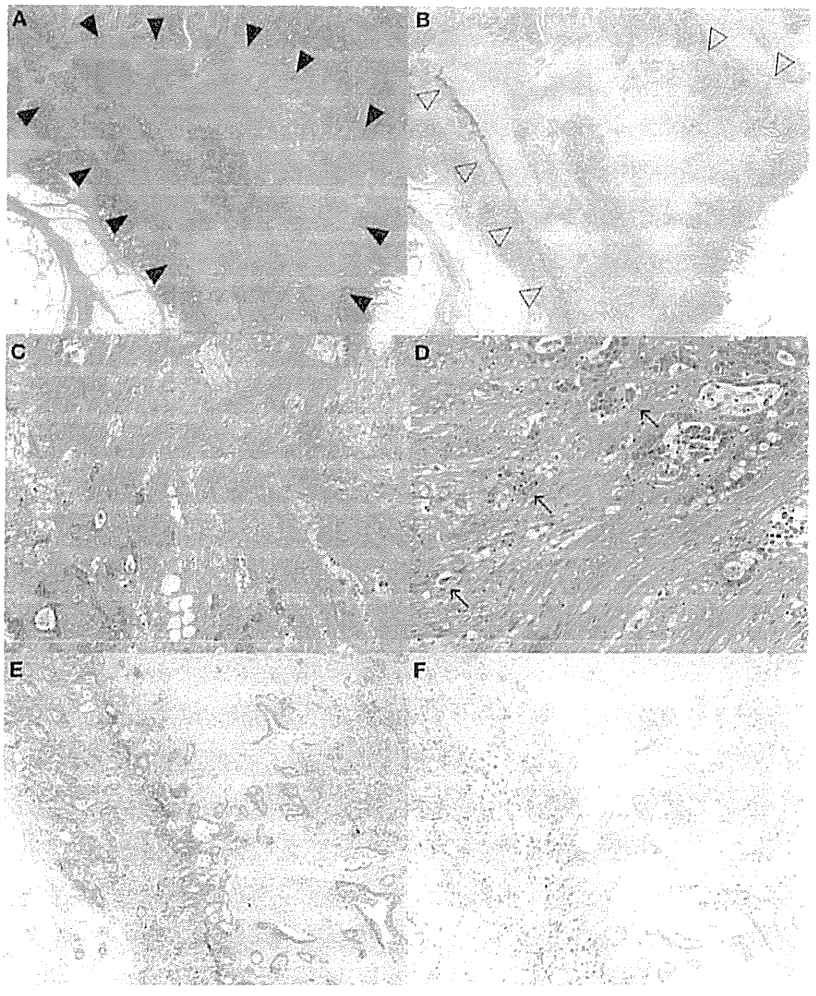

FIGURE 3 | Association of ELI, fibrotic focus, and macrophage. A scar like radiating fibrosclerotic core of a fibrotic focus [**(A)**, black arrow head] is found just beneath the peritoneal elastic lamina [**(B)**, white arrow head]. Using high-power magnification, the fibroblasts arranged in a storiform pattern **(C,D)** The distribution of CD204-positive macrophages and an association with the ELI and fibrotic focus is seen in **(E,F)**. CD204-positive macrophages are distributed along the periphery of the fibrotic focus **(E)** and along the peritoneal elastic lamina **(F)**.

DIAGNOSTIC ASSISTANCE OF ASSESSING SEROSAL INVASION USING ELASTICA STAINING: ELASTIC LAMINAL INVASION OF COLON CANCER

Special staining and immunohistochemical staining techniques may also be useful for making objective diagnoses of serosal involvement. The peritoneal elastic lamina lies just beneath the subserosal layer. This structure can be visualized using elastica stainings or elastin immunostaining. Although tumor invasion beyond the peritoneal elastic lamina (also known as ELI) is not equal to serosal involvement, based on the position, the presence of deep invasion near the peritoneal surface can be estimated. Recently, the diagnostic utility of ELI as a prognostic marker has been reported. Shinto et al. (2004) firstly reported the utility of ELI for determining high risk patients with pT3 colorectal cancers. They found that pT3 colorectal cancers with ELI (which they termed "pT3 deep") had similar clinical outcomes to patients with pT4 tumors. Their data was confirmed in our series. On the other hand, the peritoneal elastic lamina is anatomically, the deepest structure from muscular layer. Therefore ELI can only be regarded as a surrogate marker for deep tumor invasion. In this review, we analyzed our previously reported record of 304 patients with curatively resected Stage II or III colonic cancers (Kojima et al., 2010). Using a concordance probability, we evaluated the discriminatory power of ELI for over-all survival and compared that of the depth of tumor invasion. The depth of tumor invasion was measured from the outer border of the muscular layer (Shirouzu et al., 2011). As shown in **Table 1**, although the concordance probability of ELI was slightly lower than that for the lymph node metastasis, it was much larger than that for the depth of invasion. Therefore, we showed that ELI itself has at least diagnostic utility with more strong predictive power of over-all survival than the depth of tumor invasion. Rather, based on the histological features mentioned above, we estimated that the ELI microenvironment may be capable of promoting tumor progression. Elastin immunostaining was reported not to be more sensitive than elastica staining

Table 1 | Estimated concordance probability.

Variables	Concordance probability	SE
Elastic laminal invasion	0.6038	0.0272
Depth of tumor invasion	0.5510	0.0274
Lymph node metastasis	0.6117	0.0276

(Stewart et al., 2007a). Elastica staining is relatively inexpensive and stable method. The detailed examination of vascular invasion is also possible. Therefore this special staining technique can be used as a routine staining method (Abdulkader et al., 2006). Similar to lung cancer, cases with ELI can be classified as different pT entities (Puppa et al., 2011). On the other hand, the peritoneal elastic lamina does not completely cover the colonic wall. The thickness of the elastic lamina also differs depending on the anatomical site (Knudsen, 1991). This fact prompted us, in our previous study, to identify the delicate elastic lamina clearly by following it from one area to another using multiple elastica stainings. And this method made us possible to follow delicate elastic lamina. However, our method may not be practical. And peritoneal elastic lamina may not be detected in more cases in the routine practice (Canney et al., 2012). Furthermore, the staining method used for elastica staining and the number of sections that are stained have not been standardized. We are not yet sure how many slides with elastica staining are needed for a consistent ELI diagnosis. Standard recommendations for the assessment of ELI and serosal invasion are needed for consistent pathological classification.

FUTURE PERSPECTIVES AND HYPOTHESES

HISTOLOGICAL FEATURE OF ELI MAY LEAD TO BIOLOGICAL CONCEPTS INVOLVING THE CANCER MICROENVIRONMENT

Pathologists can make hypothesis based on histological findings. The presently reported histological features of the ELI area of the tumor may stimulate the imagination of pathologists, possibly leading to medical innovations. Based on our histological findings of fibroinflammation and tumor budding, new hypotheses are likely to be formulated.

FIBROINFLAMMATION AND THE MICROENVIRONMENT OF THE ELI TUMOR AREA

In addition to the histological features of fibroinflammation in the ELI area, we showed that ELI is strongly associated with distant metastasis, statistically. We have speculated that cancer cells perforate the visceral peritoneum, inducing peritoneal dissemination. However, based on our findings, we speculated that the subserosal microenvironment may provide a special means of actively inducing tumor metastasis. We have found a few previous reports that may available for proving our hypothesis. First, colonic subserosal or other subperitoneal fibroblasts have been cultured *in vitro*. These fibroblasts reportedly produce MCP-1 and VEGF in response to some biological stimuli, such as TGF-beta, IL-1beta, and TNF-alpha, or in response to physiological stimulation such as hypoxia (Witowski et al., 2001; Hirahara et al., 2004; Osada et al., 2009). We speculated that these features of subserosal fibroblast may be associated with the promotion of tumor metastasis in patients with colon cancer. Enhanced reactivity in response to stimuli may be associated with macroscopic indentation or histological fibrosis, and subsequently cancer metastasis. We would like to stress that despite the above-mentioned pathological and biological data, study on the interaction between cancer cells and peritoneal fibroblasts are very rare.

BIOLOGICAL TOPICS INCLUDING THE TUMOR MICROENVIRONMENT, CANCER STEM CELLS, AND EMT, THAT MAY BE RELEVANT TO OUR PATHOLOGICAL FINDINGS

We often see tumor budding foci in the ELI area. The microenvironment of ELI can be enriched by chemokines, cytokines, or growth factors, which may induce morphological alterations of the tumor cells (Klampfer, 2011). Budding cells have been reported to share a common phenotype with cancer stem cells or the epithelial mesenchymal transition (Brabletz et al., 2005; Kalluri and Weinberg, 2009). Both concepts are largely biological and we are not sure whether these biological concepts can be accurately compared with pathological morphological concepts, even using the expressions of cancer stem cells or EMT markers (Kojima et al., 2008). However, we now know that budding cells are enriched in the ELI. Therefore, by investigating reciprocal interactions between cancer cells and subserosal fibroblasts, we may be able to estimate the ELI microenvironment and the relevance between tumor budding and the EMT or cancer stem cells.

CONCLUDING REMARKS

We have reflected on the pathological history, examined the current status and problems, and provided future perspectives based on presently available data. We wish to mention that our current clinicopathological works have been supported by the brilliant work of many of our senior pathologists. We believe that an accurate review and recognition of this history will lead to further pathological and biological works for cancer patients.

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REFERENCES

Abdulkader, M., Abdulla, K., Rakha, E., and Kaye, P. (2006). Routine elastic staining assists detection of vascular invasion in colorectal cancer. *Histopathology* 49, 487–492.

Astler, V. B., and Coller, F. A. (1954). The prognostic significance of direct extension of carcinoma of the colon and rectum. *Ann. Surg.* 139, 846–852.

Brabletz, T., Jung, A., Spaderna, S., Hlubek, F., and Kirchner, T. (2005). Opinion: migrating cancer stem cells – an integrated concept of malignant tumour progression. *Nat. Rev. Cancer* 5, 744–749.

Canney, A. L., Kevans, D., Wang, L. M., Hyland, J. M., Mulcahy, H. E., O'Donoghue, D. P., et al. (2012). Stage II colonic adenocarcinoma: a detailed study of pT4N0 with emphasis on peritoneal involvement and the role of tumor budding. *Histopathology* 61, 488–496.

Cawthorn, S. J., Parums, D. V., Gibbs, N. M., A'Hern, R. P., Caffarey, S. M., Broughton, C. I., et al. (1990). Extent of mesorectal spread and involvement of lateral resection margin as prognostic factors after

surgery for rectal cancer. *Lancet* 335, 1055–1059.

Compton, C. C. (2003). Colorectal carcinoma: diagnostic, prognostic, and molecular features. *Mod. Pathol.* 16, 376–388.

Dukes, C. E. (1932). The classification of cancer of the rectum. *J. Pathol. Bacteriol.* 35, 323–358.

Hirahara, I., Ogawa, Y., Kusano, E., and Asano, Y. (2004). Activation of matrix metalloproteinase-2 causes peritoneal injury during peritoneal dialysis in rats. *Nephrol. Dial. Transplant.* 19, 1732–1741.

Inomata, M., Ochiai, A., Sugihara, K., Moriya, Y., Yamaguchi, N., Adachi, Y., et al. (1998). Macroscopic features at the deepest site of tumor penetration predicting liver metastases of colorectal cancer. *Jpn. J. Clin. Oncol.* 28, 123–128.

Jass, J. R., Love, S. B., and Northover, J. M. (1987). A new prognostic classification of rectal cancer. *Lancet* 1, 1303–1306.

Kalluri, R., and Weinberg, R. A. (2009). The basics of epithelial-mesenchymal transition. *J. Clin. Invest.* 119, 1420–1428.

Kirklin, J. W., Dockerty, M. B., and Waugh, J. M. (1949). The role of the peritoneal reflection in the prognosis of carcinoma of the rectum and sigmoid colon. *Surg. Gynecol. Obstet.* 88, 326–331.

Klampfer, L. (2011). Cytokines, inflammation and colon cancer. *Curr. Cancer Drug Targets* 11, 451–464.

Klimstra, D. S., Modlin, I. R., Adsay, N. V., Chetty, R., Deshpande, V., Gönen, M., et al. (2010). Pathology reporting of neuroendocrine tumors: application of the Delphic consensus process to the development of a minimum pathology data set. *Am. J. Surg. Pathol.* 34, 300–313.

Knudsen, P. J. (1991). The peritoneal elastic lamina. *J. Anat.* 177, 41–46.

Kojima, M., Ishii, G., Atsumi, N., Fujii, S., Saito, N., and Ochiai, A. (2008). Immunohistochemical detection of CD133 expression in colorectal cancer: a clinicopathological study. *Cancer Sci.* 99, 1578–1583.

Kojima, M., Ishii, G., and Ochiai, A. (2011). In response. *Am. J. Surg. Pathol.* 35, 468.

Kojima, M., Nakajima, K., Ishii, G., Saito, N., and Ochiai, A. (2010). Peritoneal elastic laminal invasion of colorectal cancer: the diagnostic utility and clinicopathologic relationship. *Am. J. Surg. Pathol.* 34, 1351–1360.

Lockhart-Mummery, J. P. (1926–1927). Two hundred cases of cancer of the rectum treated by perineal excision. *Br. J. Surg.* 7, 110–124.

Ludeman, L., and Shepherd, N. A. (2005). Serosal involvement in gastrointestinal cancer: its assessment and significance. *Histopathology* 47, 123–131.

Mills, S. E. (2007). *Histology for Pathologists.* Philadelphia: Lippincott Williams and Wilkins.

Miyamoto, S., Boku, N., Fujii, T., Ohtsu, A., Matsumoto, S., Tajiri, H., et al. (2001). Macroscopic typing with wall stricture sign may reflect tumor behaviors of advanced colorectal cancers. *J. Gastroenterol.* 36, 158–165.

Morris, E. J., Maughan, N. J., Forman, D., and Quirke, P. (2007). Who to treat with adjuvant therapy in Dukes B/stage II colorectal cancer? The need for high quality pathology. *Gut* 56, 1419–1425.

Newland, R. C., Dent, O. F., Chapuis, P. H., and Bokey, E. L. (1993). Clinicopathologically diagnosed residual tumor after resection for colorectal cancer. A 20-year prospective study. *Cancer* 72, 1536–1542.

Newland, R. C., Dent, O. F., Chapuis, P. H., and Bokey, L. (1995). Survival after curative resection of lymph node negative colorectal carcinoma. A prospective study of 910 patients. *Cancer* 76, 564–571.

Nishimura, R., Hasebe, T., Tsubono, Y., Ono, M., Sugitoh, M., Arai, T., et al. (1998). The fibrotic focus in advanced colorectal carcinoma: a hitherto unrecognized histological predictor for liver metastasis. *Virchows Arch.* 433, 517–522.

Osada, S., Hamada, C., Shimaoka, T., Kaneko, K., Horikoshi, S., and Tomino, Y. (2009). Alterations in proteoglycan components and histopathology of the peritoneum in uraemic and peritoneal dialysis (PD) patients. *Nephrol. Dial. Transplant.* 24, 3504–3512.

Puppa, G., Shepherd, N. A., Sheahan, K., and Stewart, C. J. (2011). Peritoneal elastic lamina invasion in colorectal cancer: the answer to a controversial area of pathology? *Am. J. Surg. Pathol.* 35, 465–468.

Shepherd, N. A., Baxter, K. J., and Love, S. B. (1995). Influence of local peritoneal involvement on pelvic recurrence and prognosis in rectal cancer. *J. Clin. Pathol.* 48, 849–855.

Shepherd, N. A., Baxter, K. J., and Love, S. B. (1997). The prognostic importance of peritoneal involvement in colonic cancer: a prospective evaluation. *Gastroenterology* 112, 1096–1102.

Shinto, E., Ueno, H., Hashiguchi, Y., Hase, K., Tsuda, H., Matsubara, O., et al. (2004). The subserosal elastic lamina: an anatomic landmark for stratifying pT3 colorectal cancer. *Dis. Colon Rectum* 47, 467–473.

Shirouzu, K., Akagi, Y., Fujita, S., Ueno, H., Takii, Y., Komori, K., et al. (2011). Clinical significance of the mesorectal extension of rectal cancer: a Japanese multi-institutional study. *Ann. Surg.* 253, 704–710.

Sobin, L. H., Gospondarowicz, M. K., and Wittekind, C. (2009) *TNM Classification of Malignant Tumors,* 7th Edn. New York: Wiley-Blackwell.

Stewart, C. J., Brennan, B. A., Crook, M. L., and Russell, P. (2007a). Value of elastin staining in the assessment of peritoneal implants associated with ovarian serous borderline tumours. *Histopathology* 51, 313–321.

Stewart, C. J., Morris, M., de Boer, B., and Iacopetta, B. (2007b). Identification of serosal invasion and extramural venous invasion on review of Dukes' stage B colonic carcinomas and correlation with survival. *Histopathology* 51, 372–378.

Stewart, C. J. R., Hillery, S., Platell, C., and Puppa, G. (2011). Assessment of serosal invasion and criteria for the classification of pathological (p) T4 staging in colorectal carcinoma: confusions, controversies and criticisms. *Cancers* 3, 164–181.

Van den Eynden, G. G., Colpaert, C. G., Couvelard, A., Pezzella, F., Dirix, L. Y., Vermeulen, P. B., et al. (2007). A fibrotic focus is a prognostic factor and a surrogate marker for hypoxia and (lymph) angiogenesis in breast cancer: review of the literature and proposal on the criteria of evaluation. *Histopathology* 51, 440–451.

Witowski, J., Thiel, A., Dechend, R., Dunkel, K., Fouquet, N., Bender, T. O., et al. (2001). Synthesis of C-X-C and C-C chemokines by human peritoneal fibroblasts: induction by macrophage-derived cytokines. *Am. J. Pathol.* 158, 1441–1450.

Wolpin, B. M., and Mayer, R. J. (2008). Systemic treatment of colorectal cancer. *Gastroenterology* 134, 1296–1310.

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Pancreatic Resection for Metastatic Melanoma Originating from the Nasal Cavity: A Case Report and Literature Review

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Abstract. *Metastatic pancreatic malignant melanoma is considered to be a highly aggressive neoplasm, and only few metastasectomies for lesions originating from the skin or the ocular region have been reported. We report a case of resection of pancreatic metastasis of malignant melanoma originating from the nasal cavity. An isolated pancreatic tumor was detected in a 46-year-old man who had undergone proton-beam therapy for nasal melanoma 12 months earlier. He underwent distal pancreatectomy with splenectomy and the pathological diagnosis was metastatic malignant melanoma. We review cases of malignant melanoma metastatic to the pancreas and further discuss their incidence, therapeutic strategy, and outcome of mucosal melanoma of the head and neck.*

Metastatic pancreatic tumors clinically account for fewer than 2% of all pancreatic malignancies (1, 2), and potentially resectable metastasis to the pancreas comprises 1.5-3.0% of all cases of pancreatic resection for neoplasms (2-4). Pancreatic metastases are often detected during follow-up of the primary lesion. The operative indication may differ between primary cancer and metastasis. According to a review of 243 patients with resected metastatic pancreatic tumors, the sites of origin were renal cell cancer (61.7%), colorectal cancer (7.8%), melanoma (4.9%), sarcoma (4.9%), lung cancer (3.3%), gastric cancer (3.3%), gall bladder cancer (3.3%), and breast cancer (2.5%) (1). A few decades ago, resection was usually not considered to be indicated for metastatic melanoma of the pancreas, because of multiple organ involvement and high morbidity and mortality after pancreatic surgery, but recent advances in diagnostic modalities and surgical techniques have made it acceptable. Surgical metastasectomy has the unique potential to cure the cancer or even provide palliation, whereas systemic chemotherapy for malignant melanoma only modestly improves survival. The indication for metastasectomy is limited to cases with a fair general condition, good disease control of the primary lesion, an isolated pancreatic tumor, and findings on imaging studies indicating resectable tumor. The original sites for malignant melanomas are mostly the skin of the head, neck, and lower extremities due to their frequent exposure to sunlight; however, malignant melanoma can occur in various mucosal sites where pigment cells are present. The etiopathogenesis, incidence, and clinical behavior of mucosal melanoma are considered to be different from those of skin melanoma. We present a case of pancreatic resection for metastatic melanoma originating from the nasal cavity and discuss clinical- and treatment-related issues of the condition.

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Key Words: Metastatic pancreatic tumor, melanoma of nasal cavity, distal pancreatectomy.

Case Report

A pancreatic mass was detected in a 46-year-old man during follow-up of malignant melanoma of the nasal cavity. Seventeen months earlier, he had consulted an otorhinologist with bloody rhinorrhea and was diagnosed as having malignant melanoma of the left nasal cavity, clinical stage of T3N0M0, according to the International Union Against Cancer (UICC) classification, after detailed imaging studies and biopsy (Figure 1). The tumor did not show melanin pigment macroscopically. Proton beam therapy (PBT) was delivered with a total of 60 Gy equivalents (GyE) in 15 fractions (5) and complete remission was confirmed six months after the initiation of PBT (Figure 1). However, systemic screening studies after another six months detected a solitary mass of 33×31 mm in the pancreatic body (Figure 2). Blood tests showed only slightly elevated carbohydrate antigen 19-9 levels of 57.4 U/ml (normal range <37 U/ml).

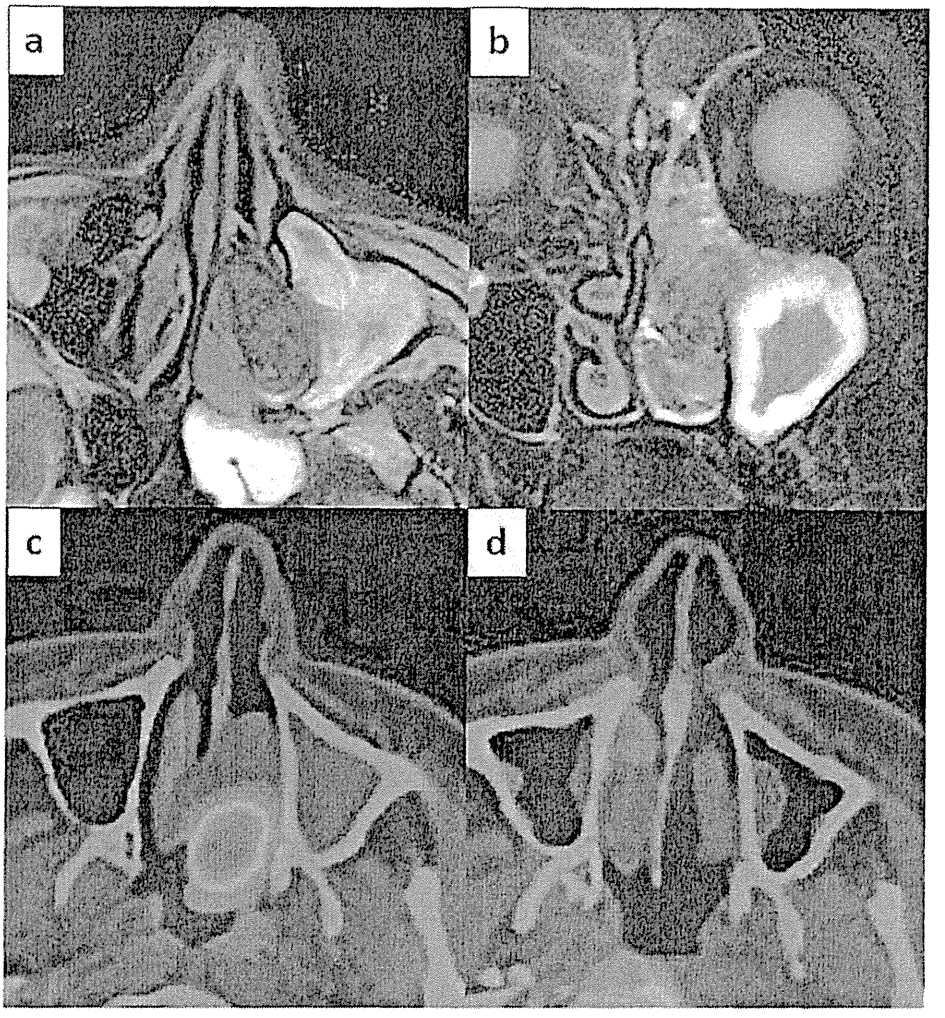

Figure 1. *Images before and after proton beam therapy (PBT). a: Initial facial Magnetic resonance imaging (MRI), axial slice. b: Initial facial MRI, coronal slice. An irregular mass in the left nasal cavity, 40×32×20 mm, extending to the ethmoid sinus, is shown. c: Pre-treatment positron emission tomography (PET) showed significant fluorodeoxyglucose accumulation (FDG) with a maximum standardized uptake value of 13.1. d: PET after PBT showed loss of FDG accumulation in the lesion.*

Solitary metastasis, well-controlled primary cancer, good general condition, and the patient's wish led to the decision to operate. Intraoperative findings did not reveal other abnormalities in the peritoneal space, and distal pancreatectomy with splenectomy as well as regional lymphadenectomy was performed. The postoperative course was uneventful and the patient was discharged on postoperative day 11. Macroscopic and histological findings with immunohistochemical profile, consistent with the findings of pre-treatment biopsy of the nasal cavity lesion, confirmed the diagnosis of metastatic melanoma of the pancreas (Figures 3 and 4). One out of 27 lymph nodes was found to be positive for metastasis. Unfortunately, metastases of the lung, skin, and intraperitoneal space were detected three months after the operation. The patient died 10 months postoperatively in spite of receiving systemic chemotherapy for recurrent disease.

Discussion

English language publications on pancreatic resection for metastatic melanoma of the pancreas are listed in Table I (3, 4, 6-25). The primary sites of malignant melanoma in the reported cases were skin, ocular region, and unknown. Although other articles reported a wide range of the interval from therapy for the primary cancer to recurrence in the

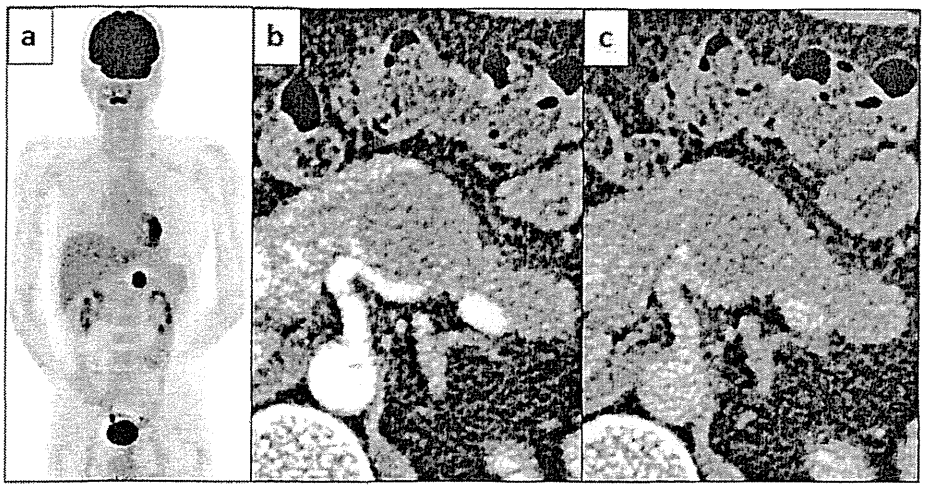

Figure 2. *Preoperative images. a: Significant fluorodeoxyglucose accumulation with a maximum standardized uptake value of 10.6 was detected in the pancreatic body on positron emission tomography. No other significant accumulation was seen. b: Computed tomography (CT) of early enhanced phase. c: CT of late enhanced phase. The tumor was located in the pancreatic body, 33×31 mm, and was oval with a clear boundary, without calcification inside. Enhancement study showed prolonged enhancement of the tumor. No main pancreatic duct dilation was observed.*

pancreas (median 6 years, range 1-24 years), the present case showed relatively early recurrence (one year) after PBT. This suggests that the natural history of malignant melanoma is variable. Our patient underwent PBT for the primary lesion because of unresectability, with concerns about cosmesis and function. Although the cancer behavior after PBT is not well-known, the biology of melanoma, especially mucosal melanoma in the head and neck, should be considered.

Regarding the incidence of mucosal melanoma of the head and neck, it accounts for only 1.4-1.7% of all cases of melanoma in Western countries, *versus* 23.3% in Japan (26, 27). Mucosal melanoma confined to the nasal cavity comprises about 4% of all sinonasal malignancies and about 80% of melanomas in the sinonasal tract (26). Mucosal melanomas generally tend to be more aggressive and have a poorer outcome than cutaneous melanoma; however, the 5-year survival rate for mucosal melanoma of the nasal cavity is 31%, which is better than that of 14-17% for malignant melanoma of the head and neck, and 0% for malignant sinus melanoma (26, 28). According to the UICC TNM classification (7th edition) of malignant melanoma of the aerodigestive tract, T1 and T2 tumors equivalent to stage I and stage II are omitted, and the emergence of cancer inidicates T3 and stage III or more, because of the highly malignant potential. The most frequent sites of distant metastases of sinonasal melanomas are lung, liver, and bones (29). These data suggest that pancreatic metastasis of nasal cavity melanoma is quite rare and is considered to indicate a poor prognosis, even though melanoma of the nasal cavity itself has a relatively fair prognosis compared to other mucosal melanomas.

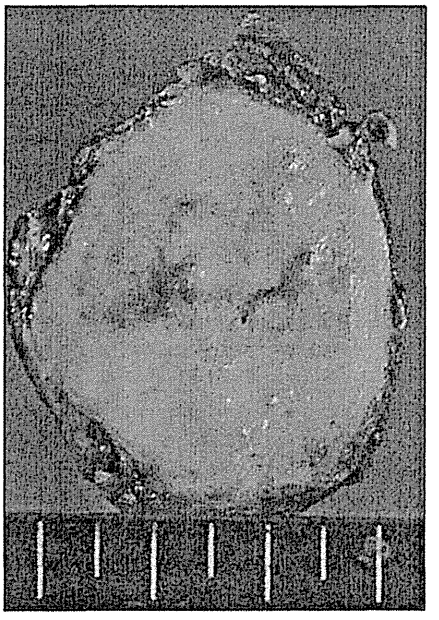

Figure 3. *Macroscopic image of tumor cut surface of resected tumor specimen. In places, the tumor circumference did not have a clear boundary from normal pancreatic parenchyma, and the cut surface was yellowish-white (amelanotic) and solid.*

With the advancement of pancreatic surgery, metastasectomy of malignant melanoma has been proven to be reasonable, although controversy exists due to its being quite an aggressive pathological condition. Reddy and Wolfgang reported the feasibility of pancreatic metastasectomy in a review of 243

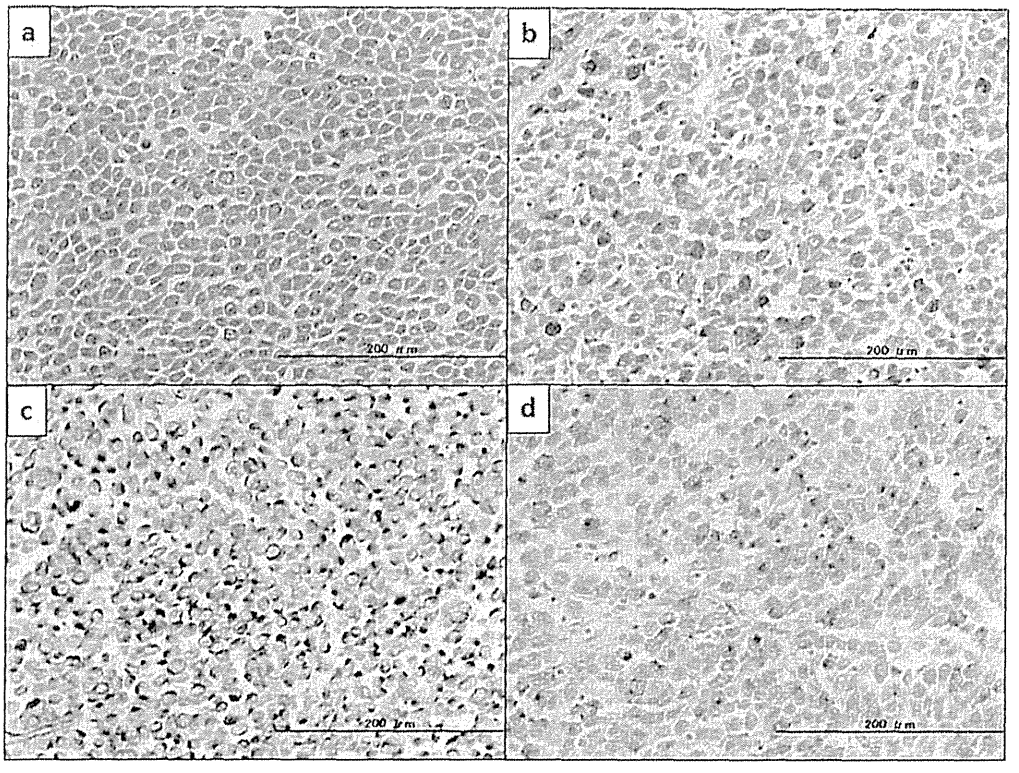

Figure 4. *Histopathological appearance of resected tumor. a: Malignant cells with round to oval nuclei and clear nucleoli showed diffuse proliferation (hematoxylin and eosin, x400). b: Positive immunohistochemical (IHC) staining of S-100 protein (×400). c: Positive IHC staining of vimentin (×400). d: Positive IHC staining with HMB-45 (×400).*

patients, which showed 38.3% morbidity and 1-2% mortality. They also reported the outcome of pancreatic resection for 11 cases of metastatic melanoma, with a median survival of 14 months (1). In a review of 234 patients with metastatic pancreatic tumors, Masetti *et al.* reported that metastases from melanoma were associated with significantly shorter survival, with a hazard ratio of 4.14, compared with that for renal cell carcinoma (30). The results of multiple single-institution studies emphasized the importance of curative resection for metastatic melanoma, with a 5-year survival rate of 15-30% and median survival time improving from 5-8 months to 15-28 months after complete metastasectomy, while patients with long-term survival have been reported (3, 10, 31-33). Prognostic factors reported to influence survival of patients with metastatic melanoma are earlier primary tumor stage, absence of intervening lymph node metastases, an interval of more than three years after resection of the primary lesion, and first metastatic sites including skin, subcutaneous tissue, lymph nodes, and lung (33).

Regarding the treatment modality for mucosal melanoma of the head and neck, complete removal with a clear surgical margin is the basic therapy for resectable lesions. As wide surgical resection in the head and neck is often difficult, radiotherapy is important for functionally and cosmetically inoperable cases. Recent reports have shown the same effectiveness in terms of local control by high-dose fractionated radiotherapy in comparison to curative surgery.

Postoperative radiotherapy was considered to be efficient for local control in patients with mucosal melanoma of the head and neck according to the studies by Krengli *et al.* (34) and Temam *et al.* (35), although they failed to show a survival benefit. Zenda *et al.* noted the safety and efficacy profiles of PBT, which provides a better dose distribution than does X-ray irradiation, with a 3-year survival rate of 58.0% for mucosal melanoma of the head and neck (5). Regarding chemotherapy, dacarbazine is often applied for disseminated malignant melanoma but the results have been disappointing, with low response rates (10-20%) and no significant improvement in survival or even lasting symptomatic relief (32, 36). Combination therapy with other drugs (cisplatin, nitrosoureas, taxanes, *etc.*) or the addition of biological therapy (interferon or interleukin-2) to standard chemotherapy results in a higher response rate but is associated with increased toxicity without significant survival benefit (31-33,

Table I. *Pancreatic resection for metastatic melanoma of pancreas collected from English literature.*

Authors (ref.)	Year	Case	Age/ Gender	Interval (years)	Primary site	Tumor size (cm)	Extrapancreatic disease	Surgery	Follow-up (months)	Outcome	Recurrence
Das Gupta and Brasfield (6)	1964	1	28/M	2	Skin	NK	NK	DP+duodenal resection	10	Dead	Yes
Johansson *et al.* (7)	1970	1	79/F	12	Ocular	NK	None	PD	11	Alive	No
Bianca *et al.* (8)	1991	1	48/M	NK	Unknown	3	Node	PD	12	Alive	Yes
Brodish and McFadden (9)	1993	1	75/F	24	Skin	5	Nodes	DP+S	8	Alive	NK
Harrison *et al.* (10)	1997	1	NK	NK	NK	NK	NK	PD	108	Alive	No
Medina-Franco *et al.* (11)	1999	1	60/M	NK	Unknown	8	Nodes	PPPD	6	Dead	Yes
Wood *et al.* (12)	2001	8	NK	NK	NK	NK	NK	NK	5-Year survival rate 37.5%		
Hiotis *et al.* (13)	2002	1	NK	NK	NK	NK	NK	PD	NK	Dead	Yes
Camp *et al.* (14)	2002	1	62/F	6	Ocular	5	Liver+nodes	DP+S with segmental hepatectomy	20	Alive	No
Nifkarjam *et al.* (15)	2003	2	45/F	12	Ocular	3	Liver	PPPD with segmental hepatectomy	6	Alive	No
			55/M	13	Ocular	NK	None	TP	7	Alive	No
Carboni *et al.* (16)	2004	1	55/F	9	Skin	8	Nodes	PD	4	Dead	Yes
Crippa *et al.* (17)	2006	1	36/F	2.7	Skin	NK	Nodes	PPPD	14	Dead	Yes
Belágyi *et al.* (18)	2006	1	28/F	6	Skin	6	Stomach, jejunum	Pancreatic enucleation, distal gastrectomy, bowel resection, and ovarian enucleation	4	Dead	Yes
Eidt *et al.* (3)	2007	4	NK	3	NK	7	NK	PPPD	12	Dead	Yes
			NK	4	NK	5	NK	PPPD	25	Dead	Yes
			NK	14	NK	5	NK	PPPD	30	Alive	No
			NK	4	NK	8	NK	PPPD	76	Alive	No
Reddy *et al.* (4)	2008	3	NK	NK	NK	Median 4	NK	NK	Median survival time 0.9 year		
Lanitis *et al.* (19)	2009	1	69/M	5	Skin	4.5	None	PD	96	Alive	No
Vagefi *et al.* (20)	2009	1	57/F	28	Ocular	2.2	Nodes	Lap-DP+S	NK	NK	NK
Sperti *et al.* (21)	2010	1	48/F	3	Unknown	2.9	Lung	DP+S and wedge resection of lung	24	Dead	Yes
He *et al.* (22)	2010	1	39/M	5	Ocular	18	None	DP+S	25	Alive	No
Goyal *et al.* (23)	2011	5	33/F	5	Skin	2	Liver and nodes	PPPD with hemihepatectomy	4.5	Dead	Yes
			50/F	3	Skin	NK	None	PPPD	15	Dead	Yes
			69/M	NK	Unknown	4.5	Nodes, spleen and stomach	DP+S with total gastrectomy	26	Dead	Yes
			73/F	22	Skin	4	None	PPPD	3	Dead	NK
			58/F	NK	Unknown	10	Duodenum and ileum	PPPD with bowel resection	11.4	Dead	NK
Portale *et al.* (24)	2011	1	43/F	7	Skin	1.7	Spleen	DP+S	NK	Alive	No
Moszkowicz *et al.* (25)	2011	1	44/F	25	Skin	1.3	None	PD	NK	NK	NK
Current	2012	1	45/M	1	Nasal cavity	3.3	Node	DP+S	10	Dead	Yes

NK, Not known; PD, pancreaticoduodenectomy; DP, distal pancreatectomy; S, splenectomy; PPPD, pylorus-preserving pancreaticoduodenectomy; TP, total pancreatectomy; Lap, laparoscopic.

36). Immunotherapy, such as, lymphokine-activated killer (LAK) cell therapy, monoclonal antibodies targeting cytotoxic T-lymphocyte antigens (CTLA-4), or onamelatucel-L (Canvaxin) is also expected, and several trials are ongoing (16, 31, 32, 37). As KIT-activating mutations in mucosal melanoma have been discovered, KIT inhibitors are considered to have potential as effective agents for this aggressive tumor (26).

In summary, the outcome of resection for pancreatic metastatic melanoma originating in the nasal cavity bore comparison with previous cases originating in the skin and ocular region, even though our case showed early recurrence. Although promising effective therapy for disseminated mucosal melanoma, especially of sinonasal origin, is not established, it is unquestionable that aggressive surgical resection produces survival benefit for properly selected patients. Careful examinations, aggressive consideration of surgical intervention, curative resection for indicated cases, and a multidisciplinary approach during follow-up of the primary lesion or even after metastasectomy, are the keys to achieving a better outcome in these patients.

References

1 Reddy S and Wolfgang CL: The role of surgery in the management of isolated metastases to the pancreas. Lancet Oncol *10(3)*: 287-293, 2009.

2 Sperti C, Pasquali C, Liessi G, Pinciroli L, Decet G and Pedrazzoli S: Pancreatic resection for metastatic tumors to the pancreas. J Surg Oncol *83(3)*: 161-166, 2003.

3 Eidt S, Jergas M, Schmidt R and Siedek M: Metastasis to the pancreas – an indication for pancreatic resection? Langenbecks Arch Surg *392(5)*: 539-542, 2007.

4 Reddy S, Edil BH, Cameron JL, Pawlik TM, Herman JM, Gilson MM, Campbell KA, Schulick RD, Ahuja N and Wolfgang CL: Pancreatic resection of isolated metastases from nonpancreatic primary cancers. Ann Surg Oncol *15(11)*: 3199-3206, 2008.

5 Zenda S, Kawashima M, Nishio T, Kohno R, Nihei K, Onozawa M, Arahira S and Ogino T: Proton beam therapy as a nonsurgical approach to mucosal melanoma of the head and neck: A pilot study. Int J Radiat Oncol Biol Phys *81(1)*: 135-139, 2010.

6 Das Gupta T and Brasfield R: Metastatic melanoma: A clinicopatholigical study. Cancer *17*: 1323-1339, 1964.

7 Johansson H, Krause U and Olding L: Pancreatic metastases from a malignant melanoma. Scand J Gastroenterol *5(7)*: 573-575, 1970.

8 Bianca A, Carboni N, Di Carlo V, Falleni M, Ferrero S, Liverani C Staudacher C, Turra G, Vergani D and Zerbi A: Pancreatic malignant melanoma with occult primary lesion. A case report. Pathologica *84(1092)*: 531-537, 1992.

9 Brodish RJ and McFadden DW: The pancreas as the solitary site of metastasis from melanoma. Pancreas *8(2)*: 276-278, 1993.

10 Harrison LE, Merchant N, Cohen AM and Brennan MF: Pancreaticoduodenectomy for nonperiampullary primary tumors. Am J Surg *174(4)*: 393-395, 1997.

11 Medina-Franco H, Halpern NB and Aldrete JS: Pancreaticoduodenectomy for metastatic tumors to the periampullary region. J Gastrointest Surg *3(2)*: 119-122, 1999.

12 Wood TF, DiFronzo LA, Rose DM, Haigh PI, Stern SL, Wanek L, Essner R and Morton DL: Does complete resection of melanoma metastatic to solid intra-abdominal organs improve survival? Ann Surg Oncol *8(8)*: 658-662, 2001.

13 Hiotis SP, Klimstra DS, Conlon KC and Brennan MF: Results after pancreatic resection for metastatic lesions. Ann Surg Oncol *9(7)*: 675-679, 2002.

14 Camp R, Lind DS and Hemming AW: Combined liver and pancreas resection with biochemotherapy for metastatic ocular melanoma. J Hepatobiliary Pancreat Surg *9(4)*: 519-521, 2002.

15 Nikfarjam M, Evans P and Christophi C: Pancreatic resection for metastatic melanoma. HPB *5(3)*: 174-179, 2003.

16 Carboni F, Graziano F, Lonardo MT, Lepiane P, Santoro R, Lorusso R, Mancini P and Santoro E: Pancreaticoduodenectomy for pancreatic metastatic melanoma. J Exp Clin Cancer Res *23(3)*: 539-543, 2004.

17 Crippa S, Angelini C, Mussi C, Bonardi C, Romano F, Sartori P, Uggeri F and Bovo G: Surgical treatment of metastatic tumors to the pancreas: a single center experience and review of the literature. World J Surg *30(8)*: 1536-42, 2006.

18 Belágyi T, Zsoldos P, Makay R, Issekutz A and Oláh A: Multiorgan resection (including the pancreas) for metastasis of cutaneous malignant melanoma. J Pancreas *7(2)*: 234-240, 2006.

19 Lanitis S, Papaioannou N, Sgourakis G, Seitz A, Zacharakis E and Karaliotas C: Prolonged survival after the surgical management of a solitary malignant melanoma lesion within the pancreas: A case report of curative resection. J Gastrointestin Liver Dis *19(4)*: 453-455, 2010.

20 Vagefi PA, Stangenberg L, Krings G, Forcione DG and Wargo JA: Ocular melanoma metastatic to the pancreas after a 28-year disease-free interval. Surgery *148(1)*: 151-154, 2010.

21 Sperti C, Polizzi ML, Beltrame V, Moro M and Pedrazzoli S: Pancreatic resection for metastatic melanoma. Case report and review of the literature. J Gastrointest Cancer *42(4)*: 302-306, 2011.

22 He MX, Song B, Jiang H, Hu XG, Zhang YJ and Zheng JM: Complete resection of isolated pancreatic metastatic melanoma: a case report and review of the literature. World J Gastroenterol *16(36)*: 4621-4624, 2010.

23 Goyal J, Lipson EJ, Rezaee N, Edil BH, Schulick R, Wolfgang CL, Hruban RH and Antonarakis ES: Surgical resection of malignant melanoma metastatic to the pancreas: case series and review of literature. J Gastrointest Cancer *243*: 431-436, 2012.

24 Portale TR, Di Benedetto V, Mosca F, Trovato MA, Scuderi MG and Puleo S: Isolated pancreatic metastasis from melanoma. Case report. G Chir *32(3)*: 135-137, 2011.

25 Moszkowicz D, Peschaud F, El Hajjam M, Saiag P and Nordlinger B: Preservation of an intra-pancreatic hepatic artery during duodenopancreatectomy for melanoma metastasis. Surg Radiol Anat *33(6)*: 547-550, 2011.

26 Mihajlovic M, Vlajkovic S, Jovanovic P and Stefanovic V: Primary mucosal melanomas: a comprehensive review. Int J Clin Exp Pathol *5(8)*: 739-753, 2012.

27 Kanetaka S, Tsukuda M, Takahashi M, Komatsu M, Niho T, Horiuchi C and Matsuda H: Mucosal melanoma of the head and neck. Exp Ther Med *2(5)*: 907-910, 2011.

28 Manolidis S and Donald PJ: Malignant mucosal melanoma of the head and neck: Review of the literature and report of 14 patients. Cancer *80(8)*: 1373-1386, 1997.

29 Thompson LD, Wieneke JA and Miettinen M: Sinonasal tract and nasopharyngeal melanomas: A clinicopathologic study of 115 cases with a proposed staging system. Am J Surg Pathol *27(5)*: 594-611, 2003.
30 Masetti M, Zanini N, Martuzzi F, Fabbri C, Mastrangelo L, Landolfo G, Fornelli A, Burzi M, Vezzelli E and Jovine E: Analysis of prognostic factors in metastatic tumors of the pancreas: A single-center experience and review of the literature. Pancreas *39(2)*: 135-143, 2010.
31 Caudle AS and Ross MI: Metastasectomy for stage IV melanoma: For whom and how much? Surg Oncol Clin N Am *20(1)*: 133-144, 2011.
32 Ollila DW, Gleisner AL and Hsueh EC: Rationale for complete metastasectomy in patients with stage IV metastatic melanoma. J Surg Oncol *104(4)*: 420-424, 2011.
33 Essner R, Lee JH, Wanek LA, Itakura H and Morton DL: Contemporary surgical treatment of advanced-stage melanoma. Arch Surg *139(9)*: 961-967, 2004.
34 Krengli M, Masini L, Kaanders JH, Maingon P, Oei SB, Zouhair A Ozyar E, Roelandts M, Amichetti M, Bosset M and Mirimanoff RO: Radiotherapy in the treatment of mucosal melanoma of the upper aerodigestive tract: Analysis of 74 cases. A Rare Cancer Network study. Int J Radiat Oncol Biol Phys *65(3)*: 751-759, 2006.
35 Temam S, Mamelle G, Marandas P, Wibault P, Avril MF, Janot F, Julieron M, Schwaab G and Luboinski B: Postoperative radiotherapy for primary mucosal melanoma of the head and neck. Cancer *103(2)*: 313-319, 2005.
36 Yang AS and Chapman PB. The history and future of chemotherapy for melanoma. Hematol Oncol Clin North Am *23(3)*: 583-597, 2009.
37 Roth TN, Gengler C, Huber GF and Holzmann D: Outcome of sinonasal melanoma: Clinical experience and review of the literature. Head Neck *32(10)*: 1385-1389, 2010.

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