Biotech Health Sci. 2015 August; 2(3): e27414. DOI: 10.17795/bhs-27414

Published online 2015 August 24. **Research Article**

The Relationship Between IMP3 Expression in Colorectal Adenocarcinoma and Clinicopathologic Findings

Fatemeh Radfar [1]; Farzad Achak [2]; Farzad Rajaei [1, 3,*]

[1]Department of Anatomy, Qazvin University of Medical Sciences, Qazvin, IR Iran
[2]Department of Pathology, Alborz University of Medical Sciences, Karaj, IR Iran
[3]Cellular and Molecular Research Centre, Qazvin University of Medical Sciences, Qazvin, IR Iran

*Corresponding author: Farzad Rajaei, Cellular and Molecular Research Centre, Qazvin University of Medical Sciences, Qazvin, IR Iran. Tel: +98-9122817421, Fax: +98-2813324970, E-mail: farzadraj@yahoo.co.uk

Received: January 27, 2015; **Revised:** June 23, 2015; **Accepted:** July 1, 2015

Background: The IMP3 is an oncofetal protein, which has been recently proposed as a diagnostic and prognostic marker in many cancers, including colorectal adenocarcinoma. The overexpression of IMP3 seems to have a correlation with patient's prognosis.
Objectives: In this study, the relationship between IMP3 expression in colorectal adenocarcinoma and clinicopathologic findings was assessed.
Patients and Methods: In this study 112 colorectal tumor paraffin blocks of Rasoul-e-Akram hospital were stained for IMP3 and slides were assessed for intensity and extent of positivity. The statistical relationships between marker expression and clinicopathologic findings (degree of differentiation, tumor size, depth of invasion and lymph nodes metastasis) were assessed. Data were analyzed by the SPSS 21 software and logistic regression and chi-square test, with p-values of less than 0.05 indicating statistical significance.
Results: Immunoreactivity pattern of IMP3 was cytoplasmic in different clinicopathologic findings. Among different clinicopathologic findings, we found a statistical relationship between tumor differentiation and IMP3 ($P = 0.047$); so that, the poorly differentiated tumors were positive for this marker. No relationship was found between tumor size, depth of invasion or lymph node involvement and IMP3.
Conclusions: IMP3 immunoreactivity was associated with poor differentiation of tumor yet not associated with tumor size depth of invasion or lymph node involvement.

Keywords: IMP3 Protein; Human; Colorectal Cancer; Clinicopathologic Finding

1. Background

Country reports of cancer registration by the cancer organization of the Iranian ministry of health, treatment and medical training in 2007 indicated that colorectal cancer in males and females with the age standardized rate (ASR) of 8.85 and 9.63 was ranked as the third and fifth most common tumor in Iran, respectively. Generally, colorectal cancer with 4493 and 4887 registered cases in years 2006 and 2007 was the forth most common tumor in Iran. According to this report 2127 females and 2679 males were diagnosed with colorectal adenocarcinoma in 2007. However, colorectal cancer is the most curable tumor of the gastrointestinal tract and most of its mortalities are preventable. Recently, IMP3 aside other oncofetal proteins has been suggested as an effective biomarker in pathogenesis and invasion of many epithelial cell cancers. This marker plays an important role in stability and trafficking of mRNA, cell growth, proliferation and migration in embryogenesis and tumoral cells, and is expressed in evolved epithelia, muscle and placenta yet is not detectable in adult tissues (1, 2). Furthermore, IMP3 or insulin like growth factor 2 mRNA binding protein 3 (IGF2BP3) is an RNA-binding oncofetal protein, which weighs 65 - 70 KD and includes 580 amino acid and four K-homolog domains and is coded by the *IMP3* gene on chromosome 7p11.5 and produces a 4350 base-pair mRNA (1, 3). Overexpression of *IMP3* (also known as L523S) has been reported in many cancers such as pancreatic carcinoma (4, 5), lung adenocarcinoma (6-8), renal cell carcinoma (9-11), hepatocellular carcinoma, malignant melanoma (12), gastric cancer (13), ovarian carcinoma (14, 15), urothelial carcinoma of bladder (16), cervical carcinoma (17, 18), and testis cancer (19). It has been suggested as a prognostic marker in some cancers including breast cancer (20), neuroblastoma (21), and cervical cancer (18). Determination of the correlation between this tumoral biomarker and clinicopathologic findings will reveal its role in pathogenesis, development and metastasis of tumors, and recognition of high-risk patients, allows their treatment by severe regimes and detailed follow-ups with short intervals, reducing their mortality. Also, this

biomarker can be used as a potential pharmacologic target in patient's treatment.

2. Objectives

With respect to the inconsistent results of previous studies in this field and the importance of colorectal carcinoma and its mortality rate in Iran, this study aimed to determine IMP3 expression in 112 colorectal cancers by immunohistochemistry assessment, and investigate its correlation with clinicopathologic findings.

3. Patients and Methods

Patients with diagnosis of colorectal cancer, who were treated with surgical resection at Rasoul-e-Akram hospital, from year 2010 to 2012, were recruited in this study. We used a computer database for collecting clinicopathologic features and omitted the cases with neoadjuvant chemoradiotherapy to minimize treatment-induced bias. The cases with a diagnosis of non-epithelial, mucinous or signet ring cancers were also excluded for homogenization of the selected cases. Finally, a total number of 112 patients with colorectal adenocarcinoma (not otherwise specified (NOS) type) were included in the immunohistochemistry (IHC) study. There were informed consents for medical research from all patients in the documented files.

3.1. Histological Evaluation

The hematoxylin and eosin stained slides of the selected cases were re-examined by a pathologist and new slides were prepared from paraffin blocks when needed. He categorized tumors as well-to-moderate or poorly differentiated adenocarcinoma. The invasion of tumor cell to different layers of colon wall (pT) was recorded by the pathologist. Tumor sizes were extracted from the computer database with one missing data. None of the cases were categorized in the PT1 category.

3.2. Immunohistochemical Evaluation

During the re-examination of H& E slides, an area with an extent of about 1 cm^2 was marked from invasive front of tumor. Tissue sections (five micrometers) were prepared from the marked areas. For antigen retrieval, deparaffinized tissue sections were rehydrated in sodium citrate buffer (pH 6) and microwaved in a pressure cooker for 10 minutes. Then, the slides were incubated for 15 minutes with peroxidase blocking reagent (Dako). Once again slides were incubated overnight in a wet chamber with a mouse monoclonal antibody against IGF2BP3 (IMP3; clone 69.1; Dako) at a dilution of 1:100. Negative tissue controls were covered by phosphate-buffered saline (PBS). For visualization, Envision Horseradish peroxidase (HRP) (Dako), diaminobenzidine (Dako), counterstaining by hematoxylin and finally mounting were respectively applied to the slides. Human placenta was used as a positive control in each work run. The IHC slides were completely examined for IMP3 immunoreactivity by microscopy and were scored for the extent of immunoreactivity as zero (0%), one (1% - 25%), two (26% - 50%), three (51% - 75%), and four (76% - 100%), depending on the percentage of positive tumoral cells. Staining intensity for Imp3 was scored as zero (negative staining), one (weak staining), two (moderate staining), and three (strong staining). The sums of staining extent and intensity were utilized for making the final scores. Based on the overall scores, IHC slides were categorized into two groups: zero to four (negative staining) and five to eight (positive staining).

3.3. Statistical Analysis

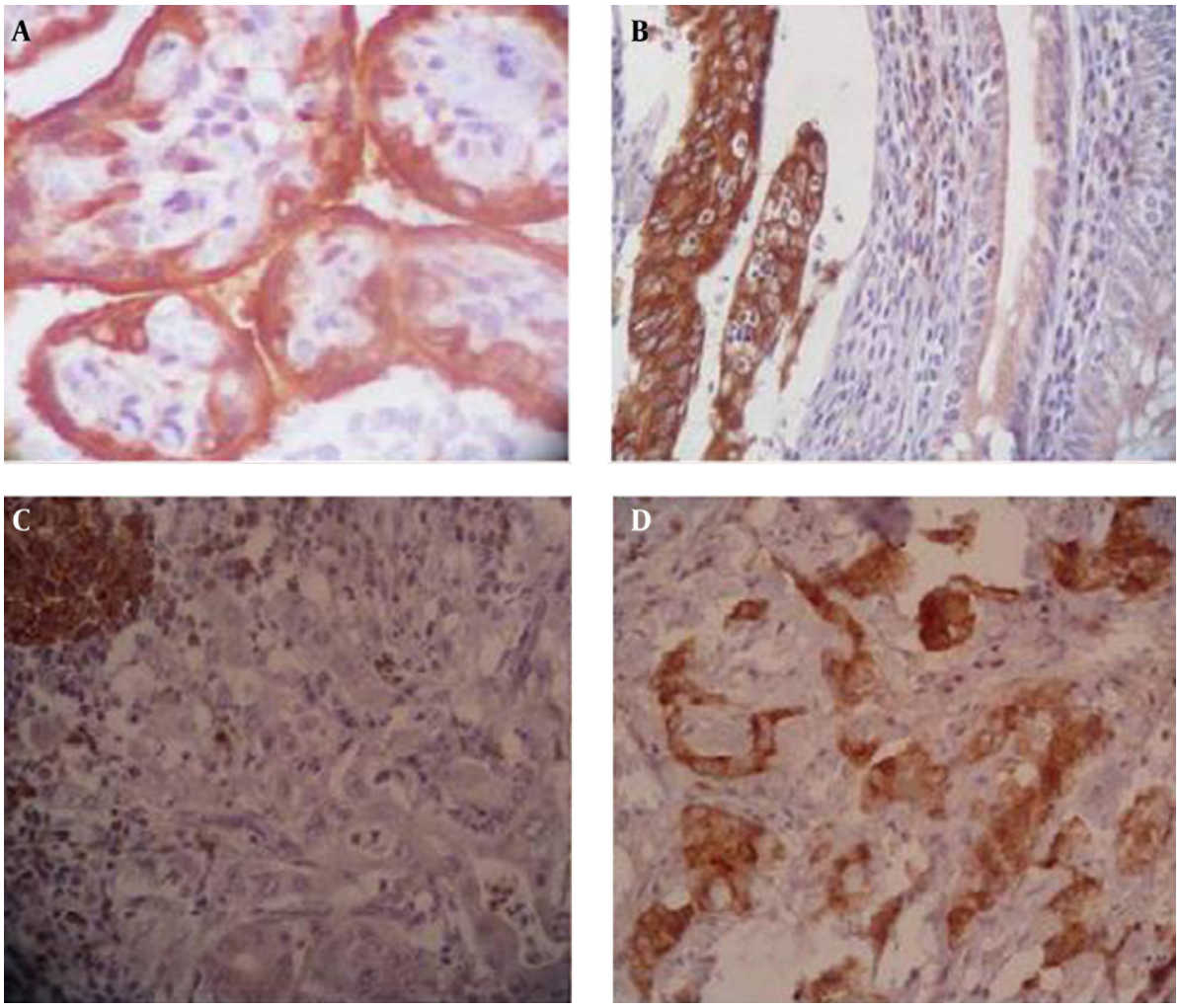
Statistical analysis including logistic regression and chi-square were carried out using the SPSS software version 21. All P values were two-tailed and considered statistically significant when less than 0.05. The hypothesis was that IMP3 immunoreactivity was associated with tumor differentiation, size, and depth of invasion or lymph nodes involvement. There was only a single data missing for tumor size, which was confirmed to have not affected the results.

4. Results

Among the database of 112 colorectal cases at Rasoul-e-Akram hospital recruited to our prospective cohort study, 56 (50%) cases showed positive immunoreactivity for IMP3 with the IHC staining method. Figure 1 shows the immunoreactivity of IMP3 in different normal and tumoral colonic tissues. In our study the pattern of staining for IMP3 in positive tumoral cells was cytoplasmic. All adjacent colonic mucosa present in the slides were negative or weakly positive. Interestingly, lymphocytes of germinal centers in mucosa-associated lymphoid tissues were IMP3 positive, acting as a positive internal control in some cases in our IHC study. Table 1 demonstrates the clinicopathologic characteristics of the 112 colorectal patients treated by surgical resection. The mean of tumor maximum diameter was 5.2 cm with standard deviation (SD) of 2.3 cm. Most of the patients (89 cases) were categorized in the pT3 group with no pT1 category (Table 2). Sixty-one (54.5%) patients had regional lymph nodes metastasis. There were only 10 high-grade (poorly differentiated) colorectal tumors and most of the cases (91%) were categorized in the low-grade group.

As it is shown, only poorly differentiation of colorectal tumor is borderline associated with IMP3 positivity (P = 0.047). We did not find any significant association between IMP3 immunoreactivity and other clinicopathologic features including tumor size, depth of invasion (pT) or lymph node metastasis (pN).

Figure 1. Immunohistochemistry Staining for IMP3



A, human placenta as positive control; the pattern of staining was cytoplasmic. B, well differentiated colorectal adenocarcinoma (left side) aside normal colonic mucosa (right side), which was IMP3 negative. C, lymphocytes of germinal center of mucosa associated lymphoid tissue (upper left) were positive for IMP3; the infiltrative tumoral cells were IMP3 negative in this case. D, IMP3 positive tumoral cells in a high-grade colorectal adenocarcinoma.

Table 1. Association of IMP3 Immunoreactivity With the Assessed Clinicopathologic Features [a]

Clinicopathologic Findings	Patients
All cases	112 (100)
Tumor diameter, cm	5.2 ± 2.3
Tumor Depth of Invasion, pT	
T1	0 (0)
T2	14 (12.5)
T3	89 (79.5)
T4	9 (8)
Lymph Nodes Metastasis, pN	
N+	61 (54.5)
N-	51 (45.5)
Tumor Differentiation	
Well to moderate differentiation, low grade	102 (91.1)
Poor differentiation, high grade	10 (8.9)

[a] Data are presented as No. (%) or mean ± SD.

Table 2. Correlation of IMP3 Expression and Clinicopathologic Features in 112 Colorectal Adenocarcinoma Cases [a]

Characteristics	IMP3 Positive	IMP3 Negative	P Value
Tumor size, cm			0.08
> 5	28 (25.2)	30 (27)	
< 5	27 (24.3)	26 (23.4)	
Tumor depth of invasion, pT			0.07 [b]
T1	0 (0)	0 (0)	
T2	6 (5.3)	8 (7.1)	
T3	46 (41.07)	43 (38.39)	
T4	4 (3.5)	5 (4.49)	
Lymph nodes metastasis, pN			0.8 [c]
Node negative	31 (27.6)	30 (26.7)	
Node positive	25 (22.4)	26 (23.4)	
Tumor differentiation			0.047
Well to moderate differentiation, low grade	48 (42.8)	54 (48.2)	
Poor differentiation, high grade	8 (7.1)	2 (1.7)	

[a] Data are presented as No. (%).
[b] T1+T2 vs. T3+T4.
[c] N negative vs. N positive.

5. Discussion

It has been reported that IGF2BP3 is a prognostic and diagnostic biomarker in many tumoral tissues including colorectal adenocarcinoma (22-25). We stained 112 formalin fixed paraffin embedded colorectal tumor tissues (NOS type) with an immunohistochemistry method and showed that 50% of these tumors were cytoplasmically positive for IMP3. We found an association between IGF2BP3 positivity and high-grade tumors. It was implied that IMP3 expression could mark a group of colorectal tumors with potential aggressive behavior. In the present study, similar to previous researches, we found no immunoreactivity in normal colon tissues, which could be used for diagnosis of colonic mass biopsy. As indicated by the study of Lochhead et al. (22), we also showed that lymphocytes of germinal centers in mucosa-associated lymphoid tissues were always positive for IMP3 as an oncofetal cytoplasmic protein, which can be troublesome for the diagnosis of crushed tumor tissue biopsy. Also, our results showed a relationship between the grade of the tumor differentiation and IMP3 immunoreactivity, while this relationship was not found by the studies of Lin et al. (23), Li et al. (24) and Yuan et al. (25). Consistent with the study of Lochhead et al. (22) and Lin et al. (23) and in contrast with the research of Yuan et al. (25), there was no relationship between tumor size and the grade of tumor differentiation in the present study. In contrast with the study of Lochhead et al. (22) and Lin et al. (23) an insignificant correlation between the depth of tumor and lymph node involvement was found. Table 3 compares our results with a few previous important researches. As it can be seen, all of these previous studies used tissue microarray for the IHC study that shows only a small area of tumors. This difference could be due to the removal of mucinous and signet-ring cell colorectal tumors in our study and the evaluation of the depth of the tumor rather than the tumor stage in our study. Another possible reason for this difference is the limited number of samples in the present study. In conclusion, the present study showed that IMP3 immunoreactivity was associated with poor differentiation of tumors, yet not associated with tumor size depth of invasion or lymph node involvement.

Table 3. Comparison of the Results of the Present Study With a Few Previous Important Researches [a]

Research	Case Number	Method	Tumor Size	Lymph Node Metastasis	Tumor Differentiation	Tumor Depth of Invasion
Present study	112	IHC	No	No	Yes	No
Lin et al. (23)	186	TMA	No	Yes	No	Yes
Lochhead et al. (22)	671	TMA	No	Yes	Yes	Yes
Li et al. (24)	203	TMA	No	Yes	No	Yes
Yuan et al. (25)	186	TMA	Yes	Yes	No	Yes

[a] Abbreviations: IHC, immunohistochemistry; and TMA, tissue microarray.

Acknowledgements

We would like to thank the deputy of the research department of Qazvin University of Medical Sciences for funding the present study and also Mrs Sharzavi for her sincere Cooperation.

Authors' Contributions

Study concept and design: Farzad Achak and Farzad Rajaei. Acquisition of data: Fatemeh Radfar. Analysis and interpretation of data: Farzad Achak. Drafting of the manuscript: Fatemeh Radfar, Farzad Achak and Farzad Rajaei. Critical revision of the manuscript for important intellectual content: Farzad Rajaei. Statistical analysis: Farzad Achak. Administrative, technical, and material support: Farzad Rajaei. Study supervision: Farzad Rajaei.

Funding/Support

This study was financially supported by the Qazvin University of Medical Sciences, Qazvin, IR Iran.

References

1. Mueller-Pillasch F, Pohl B, Wilda M, Lacher U, Beil M, Wallrapp C, et al. Expression of the highly conserved RNA binding protein KOC in embryogenesis. *Mech Dev.* 1999;**88**(1):95–9.
2. Nielsen J, Christiansen J, Lykke-Andersen J, Johnsen AH, Wewer UM, Nielsen FC. A family of insulin-like growth factor II mRNA-binding proteins represses translation in late development. *Mol Cell Biol.* 1999;**19**(2):1262–70.
3. Findeis-Hosey JJ, Xu H. The use of insulin like-growth factor II messenger RNA binding protein-3 in diagnostic pathology. *Hum Pathol.* 2011;**42**(3):303–14.
4. Schaeffer DF, Owen DR, Lim HJ, Buczkowski AK, Chung SW, Scudamore CH, et al. Insulin-like growth factor 2 mRNA binding protein 3 (IGF2BP3) overexpression in pancreatic ductal adenocarcinoma correlates with poor survival. *BMC Cancer.* 2010;**10**:59.
5. Yantiss RK, Woda BA, Fanger GR, Kalos M, Whalen GF, Tada H, et al. KOC (K homology domain containing protein overexpressed in cancer): a novel molecular marker that distinguishes between benign and malignant lesions of the pancreas. *Am J Surg Pathol.* 2005;**29**(2):188–95.
6. Simon R, Bourne PA, Yang Q, Spaulding BO, di Sant'Agnese PA, Wang HL, et al. Extrapulmonary small cell carcinomas express K homology domain containing protein overexpressed in cancer, but carcinoid tumors do not. *Hum Pathol.* 2007;**38**(8):1178–83.
7. Sojka KM, Spaulding BS, Nielsen GK, Dinnogen SA, Welcher R. Immunoreactivity of anti-L523S on normal and malignant lung pleural tissue biopsies. *Mod Pathol.* 2006;**18**:288.
8. Xu H, Bourne PA, Spaulding BO, Wang HL. High-grade neuroendocrine carcinomas of the lung express K homology domain containing protein overexpressed in cancer but carcinoid tumors do not. *Hum Pathol.* 2007;**38**(4):555–63.
9. Jiang Z, Chu PG, Woda BA, Liu Q, Balaji KC, Rock KL, et al. Combination of quantitative IMP3 and tumor stage: a new system to predict metastasis for patients with localized renal cell carcinomas. *Clin Cancer Res.* 2008;**14**(17):5579–84.
10. Jiang Z, Chu PG, Woda BA, Rock KL, Liu Q, Hsieh CC, et al. Analysis of RNA-binding protein IMP3 to predict metastasis and prognosis of renal-cell carcinoma: a retrospective study. *Lancet Oncol.* 2006;**7**(7):556–64.
11. Hoffmann NE, Sheinin Y, Lohse CM, Parker AS, Leibovich BC, Jiang Z, et al. External validation of IMP3 expression as an independent prognostic marker for metastatic progression and death for patients with clear cell renal cell carcinoma. *Cancer.* 2008;**112**(7):1471–9.
12. Pryor JG, Bourne PA, Yang Q, Spaulding BO, Scott GA, Xu H. IMP-3 is a novel progression marker in malignant melanoma. *Mod Pathol.* 2008;**21**(4):431–7.
13. Jeng YM, Wang TH, Lu SH, Yuan RH, Hsu HC. Prognostic significance of insulin-like growth factor II mRNA-binding protein 3 expression in gastric adenocarcinoma. *Br J Surg.* 2009;**96**(1):66–73.
14. Gu L, Shigemasa K, Ohama K. Increased expression of IGF II mRNA-binding protein 1 mRNA is associated with an advanced clinical stage and poor prognosis in patients with ovarian cancer. *Int J Oncol.* 2004;**24**(3):671–8.
15. Kobel M, Xu H, Bourne PA, Spaulding BO, Shih Ie M, Mao TL, et al. IGF2BP3 (IMP3) expression is a marker of unfavorable prognosis in ovarian carcinoma of clear cell subtype. *Mod Pathol.* 2009;**22**(3):469–75.
16. Sitnikova L, Mendese G, Liu Q, Woda BA, Lu D, Dresser K, et al. IMP3 predicts aggressive superficial urothelial carcinoma of the bladder. *Clin Cancer Res.* 2008;**14**(6):1701–6.
17. Mhawech-Fauceglia P, Herrmann FR, Rai H, Tchabo N, Lele S, Izevbaye I, et al. IMP3 distinguishes uterine serous carcinoma from endometrial endometrioid adenocarcinoma. *Am J Clin Pathol.* 2010;**133**(6):899–908.
18. Lu D, Yang X, Jiang NY, Woda BA, Liu Q, Dresser K, et al. IMP3, a new biomarker to predict progression of cervical intraepithelial neoplasia into invasive cancer. *Am J Surg Pathol.* 2011;**35**(11):1638–45.
19. Hammer NA, Hansen T, Byskov AG, Rajpert-De Meyts E, Grondahl ML, Bredkjaer HE, et al. Expression of IGF-II mRNA-binding proteins (IMPs) in gonads and testicular cancer. *Reproduction.* 2005;**130**(2):203–12.
20. Walter O, Prasad M, Lu S, Quinlan RM, Edmiston KL, Khan A. IMP3 is a novel biomarker for triple negative invasive mammary carcinoma associated with a more aggressive phenotype. *Hum Pathol.* 2009;**40**(11):1528–33.
21. Chen ST, Jeng YM, Chang CC, Chang HH, Huang MC, Juan HF, et al. Insulin-like growth factor II mRNA-binding protein 3 expression predicts unfavorable prognosis in patients with neuroblastoma. *Cancer Sci.* 2011;**102**(12):2191–8.
22. Lochhead P, Imamura Y, Morikawa T, Kuchiba A, Yamauchi M, Liao X, et al. Insulin-like growth factor 2 messenger RNA bind-

ing protein 3 (IGF2BP3) is a marker of unfavourable prognosis in colorectal cancer. *Eur J Cancer.* 2012;**48**(18):3405–13.
23. Lin L, Zhang J, Wang Y, Ju W, Ma Y, Li L, et al. Insulin-like growth factor-II mRNA-binding protein 3 predicts a poor prognosis for colorectal adenocarcinoma. *Oncol Lett.* 2013;**6**(3):740–4.
24. Li D, Yan D, Tang H, Zhou C, Fan J, Li S, et al. IMP3 is a novel prognostic marker that correlates with colon cancer progression and pathogenesis. *Ann Surg Oncol.* 2009;**16**(12):3499–506.
25. Yuan RH, Wang CC, Chou CC, Chang KJ, Lee PH, Jeng YM. Diffuse expression of RNA-binding protein IMP3 predicts high-stage lymph node metastasis and poor prognosis in colorectal adenocarcinoma. *Ann Surg Oncol.* 2009;**16**(6):1711–9.