Preoperative survivin mRNA detection in peripheral blood is an independent predictor of outcome in esophageal carcinoma

Aims: Survivin (SVV) mRNA expression levels in peripheral blood of patients with gastrointestinal malignancies change significantly during the course of treatment. We wanted to scrutinize these findings in patients with esophageal carcinoma and furthermore evaluate whether the detection of mRNA and the change in detecting ability have an association with overall survival. **Materials & methods:** Whole blood was drawn 1 day pre- and 10 days post-operatively from 62 patients with esophageal carcinoma. Tumor cells were enriched from whole blood by density-gradient centrifugation prior to extraction of total cellular RNA and subsequent direct quantitative reverse transcriptase-PCR assays. **Results:** SVV was detectable in 48 out of 62 patients (77%). Stepwise multivariate Cox linear regression models demonstrated a significant and independent association of measured SVV with overall survival (6.6 exp[b]; 95% CI: 1.97–22.12; p = 0.002). Increased SVV levels after the operation were linked to shorter overall survival (p = 0.04). **Conclusion:** Preoperative SVV expression levels appear to be associated with overall survival in patients with esophageal cancers. Increasing levels could potentially indicate a higher risk for shorter overall survival and therefore demand adapted treatment modalities.

KEYWORDS: circulating tumor cells ■ esophageal cancer ■ outcome ■ survivin ■ SVV

Andreas-Claudius Hoffmann[1†], Daniel Vallböhmer[2], Peter Grimminger[2], Ralf Metzger[2], Klaus L Prenzel[2], Arnulf H Hoelscher[2] & Jan Brabender[2]

[†]Author for correspondence:
[1]Department of Medicine (Cancer Research), Molecular Oncology Risk-Profile Evaluation (MORE), West German Cancer Center, University Hospital Essen, Hufelandstrasse 55, Essen, 45147, Germany
Tel.: +49 201 723 85522
Fax: +49 201 723 5733
Email: hoffmann@more-oncology.com
[2]Department of General, Visceral & Cancer Surgery, University of Cologne, Cologne, Germany

Esophageal cancer shows a poor overall survival rate. If treated under curative intentions, the main contributing factors to prolonged progression-free and overall survival are tumor-free resection (R0 resection) [1,2], lymph node involvement [3] and complete histopathologic response after applied multimodality treatment [4–6]. The treatment of extensive disease consists of chemotherapy with concurrent radiotherapy or chemotherapy alone, dependent on the histology and stage of the tumor [7]. A common challenge when administering chemotherapy is making the right choice of regimen and dosage, especially since it has recently been discussed whether preoperative radiochemotherapy, despite its obvious benefits of increasing the rates of complete tumor resection, improving local tumor control and thereby improving survival in Phase III trials, might also lead to a higher postoperative mortality rate [7–13]. Therefore, factors that would help to better estimate 5-year survival chances could eventually lead to the best treatment choice for the individual patient. To date, there have been many attempts at finding the right marker at the right time in the right tissue. Although results found with measuring gene expression in fresh, frozen or paraffinized tissue seem to be promising, the defined predictors of histopathologic response or outcome may only be accurate at the time of sampling, for instance before first-line treatment. Furthermore, biopsy techniques put the patient at additional risk owing to their invasive nature. Consequently, the best approach to monitoring the treatment of a patient would be to detect markers in peripheral blood that have an association to response or overall survival.

As previously published, we established a direct quantitative real-time RT-PCR assay (TaqMan®, Applied Biosystems, CA, USA) for measuring mRNA expression in enriched tumor cells from peripheral blood samples [14]. We used this technique to measure, among others, excision repair cross-complementing one mRNA levels in peripheral blood to predict histopathologic response to platinum-based treatment regimes in esophageal cancer [15]. Survivin (SVV) is an inhibitor of apoptosis and is frequently expressed at high levels in various human cancers [16,17]. Several study groups have demonstrated that SVV mRNA expression is linked to outcome of patients with various cancers [18,19]. We therefore wanted to test whether preoperative SVV expression in peripheral blood is associated with outcome.

Materials & methods

■ Study population, demographic data & staging procedures

We drew blood samples from 62 patients with esophageal cancers (EC) with a median age of 61 years at time of operation who were scheduled

future medicine part of fsg

for surgical resection. A total of 25 out of 62 (40.3%) patients had squamous cell carcinoma and 37 out of 62 (59.7%) had adenocarcinoma. A total of 24 out of 62 (38.7%) patients received neoadjuvant chemoradiation followed by surgical resection. All patients received treatment at the University Hospital of Cologne, Germany, between August 2002 and August 2004, and data were collected consecutively. Neoadjuvant multimodality treatment was carried out according to regimens previously published by Vallböhmer and others [20]. Demographic, clinical and histopathologic parameters are as displayed in Table 1. The median follow-up time was 3 years. We obtained informed consent from each patient in accordance with the requirements of our institution's board of ethics. Tumor, node, metastasis staging was performed according to the criteria of the International Union Against Cancer [21].

Tumor cell enrichment, isolation of mRNA

A total of 20 ml of whole blood was drawn 1 day preoperatively and 7–10 days post-resection using 10 ml citrate tubes (Sarstedt, Nuembrecht, Germany). After an incubation of 15 min on ice, the whole-blood sample was transferred to a 50 ml centrifugation tube containing a porous barrier and separation medium (OncoQuick®, Hexal, Frickenhausen, Germany). During a 20-min centrifugation step, blood cells and plasma were separated according to their different buoyant densities and the circulating tumor cells were enriched in a layer formed between the plasma and the separation medium. This cell fraction was then used to isolate the total cellular mRNA as extensively described by Hoffmann *et al.* in 2007 [14].

Quantitative real-time PCR

After generating cDNA using oligo (dT)18 primers and Moloney murine leukemia virus reverse transcriptase (Advantage™ Kit, Clontech Laboratories, Inc., CA, USA), direct quantitative real-time RT-PCR (TaqMan, ABI PRISM® 7900 HT Sequence Detection System®, Applied Biosystems) assays were performed in triplicate to determine SVV expression levels in peripheral blood. Owing to low amounts of tumor cells and therefore low mRNA/cDNA (generally between 2 and 4 ng/5 µl) levels, we used all the material from each run for the triplicates (5 µl per triplicate, 15 µl per sample). PCR conditions were as previously described [14]. The detection of amplified cDNA resulted in a cycle threshold (Ct) value that was inversely proportional to the amount of cDNA. The higher the ensuing Ct value, the more PCR cycles were necessary to attain detection limit, indicating less cDNA. All results are expressed as ratios between two absolute measurements (gene of interest/endogenous reference gene or β-actin) to account for loading differences.

All experiments were carried out in a blinded fashion, including steps such as collecting the samples and isolating the mRNA. Independent staff performed the statistical analysis.

Table 1. Patient characteristics.

Parameter	Number of patients (%)
Gender	
Male	53 (85.48)
Female	9 (14.52)
Histology	
Adenocarcinoma	37 (59.68)
Squamous cell carcinoma	25 (40.32)
Treatment	
Adjuvant	29 (46.77)
Neoadjuvant	24 (38.71)
Not applicable	9 (14.52)
pT category	
pT0	3 (4.84)
pT1	14 (22.58)
pT2	8 (12.90)
pT3	23 (37.10)
Not evaluated	14 (22.58)
pN category	
N-	29 (46.77)
N+	31 (50.00)
Not evaluated	2 (3.23)
c/pM category	
c/pM0	44 (70.97)
c/pM1	5 (8.06)
Not evaluated	13 (20.97)
Grading	
G1	1 (1.61)
G2	26 (41.94)
G3	15 (24.19)
Not evaluated	20 (32.26)
Residual tumor category	
R0	43 (69.35)
R1	5 (8.06)
Not evaluated	14 (22.58)

Total number of patients: 62.
Median age (years): 61 (range: 40–80).
c/pM: Distant metastasis; G: Grade of differentiation; pN: Regional lymph node metastasis; pTNML: Tumor-node-metastasis pathological classification; pT: Primary tumor expansion; R: Residual tumor category.

Statistical analysis

We used the Wilcoxon test for paired samples to test for significant changes in gene-expression values during therapy, including the pre- and post-operative measurement as paired samples. We also applied the Mann–Whitney U-Test for non-normally distributed samples to evaluate whether SVV expression levels were associated with the different therapeutic regimens (neoadjuvant or adjuvant radiotherapy/chemotherapy) and other clinicopathologic factors. SVV was tested with the Kaplan–Meier method to estimate the different associations of gene-expression levels with overall survival. We analyzed differences in survival between the high- and the low-expression group with the log-rank test. To assess whether gene-expression levels are independently associated with overall survival, we used multivariate Cox proportional hazards regression analysis with stepwise selection on SVV expression, using primary tumor expansion and other factors as covariates after adjustment for potential confounders (e.g., tumor staging, type of tumor resection and patients' age).

Using a data-mining technique provided by the SAS institute we split gene expression into high- and low-level groups based on a partition platform that recursively partitions data according to a relationship between the X- and Y- values, creating a tree of partitions. By searching all possible cuts, it finds a set of cuts of X-values (gene expression) that best predict the Y-value (clinical factor). These data splits are carried out recursively, forming a tree of decision rules until the desired fit is reached; the most significant split is determined by the largest likelihood-ratio χ^2 statistic. In either case, the split is chosen to maximize the difference in the responses between the two branches of the split. Lu and others previously used this method [22–24].

The level of significance was set to a p-value of less than 0.05. All p-values reported were based on two-sided tests. All statistical tests were performed using the Software Packages SPSS® for Windows, Version 17.0, (SPSS, IL, USA) and JMP 7.0 Software (SAS, NC, USA).

Results

In total, 48 out of 62 patients (77%) demonstrated detectable levels of SVV in peripheral blood.

Differences between groups

Using the Wilcoxon test for paired samples, SVV expression levels were differed in 30 out of 48 (63%) patients during the course of operation. A total of 17 out of 48 patients demonstrated increased SVV levels (p = 0.0001) after the operation, while 13 out of 48 (27%) declined significantly postoperatively (p = 0.0002).

The Mann–Whitney U-test demonstrated no significant difference in the detectable pre-operative SVV levels between adenocarcinoma (median: 0.058; range: 0.0–0.9826) and squamous cell carcinoma (median: 0.048; range: 0.026–0.9937; p = 0.66). Detection of SVV was also not significantly influenced by the treatment modality (p = 0.77). Neither the primary tumor expansion (pT; p = 0.74), lymph node involvement (p = 0.54), distant metastasis (p = 0.74), grade of dedifferentiation (p = 0.43) nor the residual tumor status (p = 0.13) were significantly differently associated with SVV expression levels. Interestingly, patients who did not have detectable levels of SVV demonstrated a significantly different survival time to those with detectable levels (p = 0.05).

Gene expression & survival

For survival analysis, we used the 30th percentile as a cut-off point as defined by the recursive descent partition test. In Kaplan–Meier analysis, SVV expression separated patients into two groups with distinct survival. Patients with an SVV expression level lower than the 30th percentile had a survival probability of 80% after 5 years, whereas patients demonstrating high expression levels had a survival probability of 50% (hazard ratio [HR]: 6.13; 95% CI: 1.79–21.03; p = 0.004, Figure 1). Reaching the detection limit of SVV was associated with an increased risk for shortened overall survival (HR: 5.59; 95% CI: 1.54–20.34; p = 0.01). If SVV expression levels did not increase after the operation, patients had a lower relative risk of shortened overall survival. In Kaplan–Meier analysis, this almost reached significance (HR: 0.33; 95% CI: 0.09–1.19; p = 0.09). To substantiate these findings and to examine whether SVV expression is an independent factor associated with survival, we used Cox proportional hazards regression analysis with stepwise selection in a model containing SVV expression, the primary tumor expansion, lymph node and distant metastasis, residual tumor status and survival. We looked at all patients independent of their possible confounders, such as distant metastasis, neoadjuvant chemotherapy or residual tumor status. The analysis revealed that SVV expression was the strongest independent factor, with a relative risk of 6.6 exp[b] (95% CI: 1.97–22.12; p = 0.002) of higher SVV for shorter survival. Excluding patients with distant metastasis or

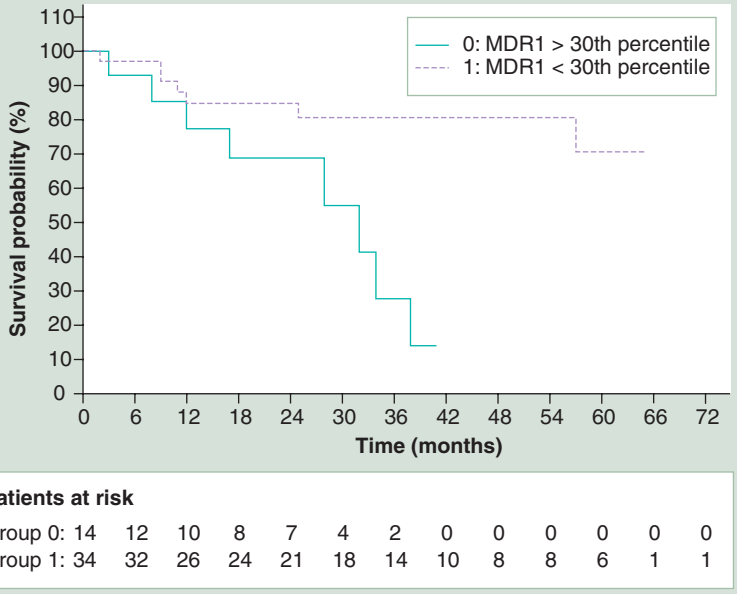

Figure 1. Kaplan–Meier plot, estimating overall survival. Differences in survival between the high (lower solid line) and the low SVV expression group were analyzed with the log-rank test.
Hazard ratio: 6.13; 95% CI: 1.79–21.03; p = 0.004.

residual tumor (as assessed by the pathologist) the relative risk for worse outcome if SVV was expressed at higher levels was 5.68 exp[b] (95% CI: 1.41–22.90; p = 0.01). Analyzing only patients who had no distant metastasis, no residual tumor and no immunohistochemically detected lymph node metastasis, higher SVV expression imposed a relative risk for shortened survival time of 16.39 exp[b] (95% CI: 2.05–131.10; p = 0.02). Higher primary tumor expansion, now included in the model, had a relative risk of 23.1 exp[b] (95% CI: 1.30–409.45; p = 0.03). Subgroup analysis revealed similar results in adenocarcinoma, as well as squamous cell carcinoma, although in adenocarcinoma 10.9 exp[b] (95% CI: 1.53–77.50; p = 0.02) SVV expression in peripheral blood seems to have a stronger association with overall survival than in squamous cell carcinoma 6.6 exp[b] (95% CI: 0.6144–70.9332; p = 0.1). Using increasing SVV expression levels in Cox regression analysis, the overall model fit was p = 0.04 with a relative risk of 4.6 exp[b] (95% CI: 0.8758–24.0046; p = 0.07) for shorter overall survival if increased SVV was detected.

Discussion

The main goal of this study was to assess whether there is an association of SVV expression in the peripheral blood of patients with esophageal carcinoma with overall survival. With regard to this goal, our data showed that high SVV expression is associated with a shortened time of survival. The implementation of Kaplan–Meier analysis and multivariate Cox proportional hazards regression analysis illustrated a significant association of SVV expression with overall survival. Furthermore, SVV expression in this patient cohort seemed to have a stronger independent association with survival than primary tumor expansion, distant metastasis, lymph node involvement and residual tumor status.

Before discussing the possible implications of the study results, we wish to emphasize that the study group was not homogeneous. It is already an established fact that patients with adenocarcinoma or squamous cell carcinoma of the esophagus have different preconditions. This may be owing to a sometimes different socioeconomic background and therefore a very diverse spectrum of associated diseases, or to different anatomical location, which demands different surgical approaches, or to dissimilar lymph node involvement and therefore varying risks for locoregional recurrence [25,26]. In fact, squamous cell and adenocarcinoma of the esophagus are hardly the same disease [4,27]. Though this may obviously be a source of bias in this study, we still think these results are of interest. As multivariate Cox proportional hazards regression and Kaplan–Meier analysis revealed, SVV kept its significant association to overall survival in all subgroups. The higher relative risk of patients with high levels of SVV in peripheral blood in adenocarcinoma, as well as in squamous cell carcinoma, whether they underwent neoadjuvant treatment or not, underlines the possible use of this gene in monitoring patients during different courses of therapy.

As previously published, we have already seen significant changes in the ability of SVV to detect different gastrointestinal tumors before and after surgery [14]. We are now able to link these changes in detection to an either decreased or increased risk of shorter overall survival. As several study groups have discussed before, SVV expression is relatively higher in tumor than in nonmalignant cells [16,17,28]. This may imply that SVV expression is associated with the amount of circulating tumor cells in peripheral blood, especially since we used a buoyant density gradient to enrich peripheral blood mononuclear cells before extracting the mRNA. Therefore, we speculate that the strong independent association of detected SVV to overall survival relates to the association of circulating tumor cells with outcome as already described in gastrointestinal and other cancers [29–31]. Although applying more specific enrichment methods, such as immunomagnetic

bead selection of tumor cells, may increase specificity and perhaps sensitivity, these approaches are more time consuming and have pitfalls of their own [32]. In our opinion, using peripheral blood mononuclear cells has the advantage of being more specific than using whole blood but is also feasible with limited laboratory equipment, which could be helpful when validating these results prospectively in a multicenter approach with larger patient groups. Recently, we were able to use this technique to examine whether response to neoadjuvant treatment is also associated with SVV levels [33]. Furthermore, other study groups did find similar associations of SVV detection in peripheral blood with overall survival and shortened time to relapse or progression [34–37].

Despite some previous findings, we did not find a higher SVV level associated with a favorable outcome, but rather to shortened overall survival [38]. We hypothesize that this may be owing to differently regulated anti-apoptosis pathways in circulating tumor cells and the primary tumor cells. Migration is only possible if programmed cell death, normally induced by an ectopic environment, is inactivated [39]. SVV expression detected in tumor tissue also reflects the cell cycle activity of the tumor and surrounding stromal cells, as SVV in the tumor underlies specific cell cycle regulation [40–43]. Therefore, it is conceivable that SVV expression in circulating tumor cells is not regulated in the same way as in the primary tumor. Recently, Schwarzenbach and others demonstrated that circulating cell-free tumor DNA is highly associated to the presence of circulating tumor cells [31]. Since it is obvious that mRNA is not very stable outside the environment of the cytoplasm, detecting tumor-specific mRNA levels in peripheral blood seems to be a possible approach to detect circulating tumor cells [44,45].

Conclusion

The results of this study seem to underline the importance of detecting SVV expression in peripheral blood, especially since the results of other recently published studies on different entities came to similar conclusions. We were able to demonstrate a significant and independent association of measured SVV with overall survival (6.6 exp[b]; 95% CI: 1.97–22.12; p = 0.002). Moreover, we were able to validate previous findings of changed levels during the course of operation and could even link these changes to a significantly dissimilar overall survival. These findings, along with the results of independent study groups, seem to imply that SVV could be a useful biomarker for predicting treatment outcome and possibly overall survival; hence, detection of SVV in peripheral blood could be an important step towards personalized cancer treatment. Since we obtained these results from a nonhomogenous study group, larger studies with bigger subgroups seem to be warranted.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- Multimodality treatment of esophageal cancer is still not optimal; it is unclear whether chemoradiation, surgery or a combination of all treatment approaches are successful strategies.
- Defining subgroups that are most likely to benefit from surgery or defining those that most probably should be treated with chemoradiation could be a crucial step forwards.
- To this end, biomarkers for response prediction and assessment of shorter survival are important.

Materials & methods

- Consecutively treated patients were included in the study, after follow-up association of survivin (SVV) with overall survival was examined.
- Whether gene-expression levels were an independent factor associated with survival was elucidated by multivariate Cox regression analysis.

Results

- In total, 77% of patients demonstrated detectable SVV levels in peripheral blood.
- Detection of SVV was associated with decreased survival; moreover, an increase of SVV detection in peripheral blood was an independent risk factor for shortened survival time.

Discussion

- The results of this pilot study confirm the results of other study groups that examined SVV detection in different entities and found a significant association with response, tumor relapse and survival.
- The results underline the possible use of SVV detection as a new biomarker for personalizing cancer care.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Bibliography

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

1 Holscher AH, Vallböhmer D: [Surgical treatment of esophageal tumors including local ablation]. *Zentralbl. Chir.* 132(2), W18–W36; quiz W37–W38 (2007).

2 Lordick F, Ott K, Novotny A, Schuhmacher C, Siewert JR: [R1 resection in the surgery of upper gastrointestinal tumors: relevance and therapeutic consequences]. *Chirurg* 78(9), 792–801 (2007).

3 Metzger R, Drebber U, Baldus SE, Monig SP, Holscher AH, Bollschweiler E: Extracapsular lymph node involvement differs between squamous cell and adenocarcinoma of the esophagus. *Ann. Surg. Oncol.* 16(2), 447–453 (2009).

4 Heitmiller RF: Epidemiology, diagnosis, and staging of esophageal cancer. *Cancer Treat. Res.* 105, 375–386 (2001).

5 Schneider PM, Baldus SE, Metzger R *et al.*: Histomorphologic tumor regression and lymph node metastases determine prognosis following neoadjuvant radiochemotherapy for esophageal cancer: implications for response classification. *Ann. Surg.* 242(5), 684–692 (2005).

6 Wong R, Malthaner R: Esophageal cancer: a systematic review. *Curr. Probl. Cancer.* 24(6), 297–373 (2000).

7 Stahl M, Oliveira J: Esophageal cancer: ESMO clinical recommendations for diagnosis, treatment and follow-up. *Ann. Oncol.* 19(Suppl. 2), II21–II22 (2008).

8 Melis M, McLoughlin JM, Dean EM *et al.*: Correlations between neoadjuvant treatment, anemia, and perioperative complications in patients undergoing esophagectomy for cancer. *J. Surg. Res.* 153(1), 114–120 (2008).

9 Tsujimoto H, Ono S, Chochi K, Sugasawa H, Ichikura T, Mochizuki H: Preoperative chemoradiotherapy for esophageal cancer enhances the postoperative systemic inflammatory response. *Jpn. J. Clin. Oncol.* 36(10), 632–637 (2006).

10 Smithers BM, Cullinan M, Thomas JM *et al.*: Outcomes from salvage esophagectomy post definitive chemoradiotherapy compared with resection following preoperative neoadjuvant chemoradiotherapy. *Dis. Esophagus* 20(6), 471–477 (2007).

11 Urba SG, Orringer MB, Turrisi A, Iannettoni M, Forastiere A, Strawderman M: Randomized trial of preoperative chemoradiation versus surgery alone in patients with locoregional esophageal carcinoma. *J. Clin. Oncol.* 19(2), 305–313 (2001).

12 Swisher SG, Holmes EC, Hunt KK, Doty JE, Zinner MJ, McFadden DW: The role of neoadjuvant therapy in surgically resectable esophageal cancer. *Arch. Surg.* 131(8), 819–824; discussion 24–25 (1996).

13 Lordick F, Ebert M, Stein HJ: Current treatment approach to locally advanced esophageal cancer: is resection mandatory? *Future Oncol.* 2(6), 717–721 (2006).

14 Hoffmann AC, Warnecke-Eberz U, Luebke T *et al.*: Survivin mRNA in peripheral blood is frequently detected and significantly decreased following resection of gastrointestinal cancers. *J. Surg. Oncol.* 95(1), 51–54 (2007).

▪ **Provides the basis for this article.**

15 Brabender J, Vallböhmer D, Grimminger P *et al.*: ERCC1 RNA expression in peripheral blood predicts minor histopathological response to neoadjuvant radio-chemotherapy in patients with locally advanced cancer of the esophagus. *J. Gastrointest. Surg.* 12(11), 1815–1821 (2008).

16 Reed JC: The survivin saga goes *in vivo*. *J. Clin. Invest.* 108(7), 965–969 (2001).

▪▪ **Important paper regarding the role of survivin in cancer.**

17 Ambrosini G, Adida C, Altieri DC: A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. *Nat. Med.* 3(8), 917–921 (1997).

▪▪ **First description of survivin.**

18 Weiss C, von Romer F, Capalbo G *et al.*: Survivin expression as a predictive marker for local control in patients with high-risk T1 bladder cancer treated with transurethral resection and radiochemotherapy. *Int. J. Radiat. Oncol. Biol. Phys.* 74(5), 1455–1460 (2009).

19 Hsu KF, Lin CK, Yu CP *et al.*: Cortactin, fascin, and survivin expression associated with clinicopathological parameters in esophageal squamous cell carcinoma. *Dis. Esophagus* 22(5), 402–408 (2009).

20 Vallböhmer D, Kuhn E, Warnecke-Eberz U *et al.*: Failure in downregulation of intratumoral survivin expression following neoadjuvant chemoradiation in esophageal cancer. *Pharmacogenomics* 9(6), 681–690 (2008).

21 Sobin LH, Fleming ID: TNM classification of malignant tumors, fifth edition (1997). Union Internationale Contre le Cancer and the American Joint Committee on Cancer. *Cancer* 80(9), 1803–1804 (1997).

22 Lu KH, Patterson AP, Wang L *et al.*: Selection of potential markers for epithelial ovarian cancer with gene expression arrays and recursive descent partition analysis. *Clin. Cancer Res.* 10(10), 3291–3300 (2004).

23 Hoffman AC, Danenberg KD, Taubert H, Danenberg PV, Wuerl P: A three-gene signature for outcome in soft tissue sarcoma. *Clin. Cancer Res.* 15(16), 5191–5198 (2009).

24 Hoffmann AC, Mori R, Vallböhmer D *et al.*: High expression of HIF1a is a predictor of clinical outcome in patients with pancreatic ductal adenocarcinomas and correlated to PDGFA, VEGF, and bFGF. *Neoplasia* 10(7), 674–679 (2008).

25 Rohatgi PR, Swisher SG, Correa AM *et al.*: Comparison of clinical stage, therapy response, and patient outcome between squamous cell carcinoma and adenocarcinoma of the esophagus. *Int. J. Gastrointest. Cancer* 36(2), 69–76 (2005).

26 Bollschweiler E, Wolfgarten E, Gutschow C, Holscher AH: Demographic variations in the rising incidence of esophageal adenocarcinoma in white males. *Cancer* 92(3), 549–555 (2001).

27 Siewert JR, Ott K: Are squamous and adenocarcinomas of the esophagus the same disease? *Semin. Radiat. Oncol.* 17(1), 38–44 (2007).

28 Khan Z, Tiwari RP, Mulherkar R *et al.*: Detection of survivin and p53 in human oral cancer: correlation with clinicopathologic findings. *Head Neck* 31(8), 1039–1048 (2009).

29 Cohen SJ, Alpaugh RK, Gross S *et al.*: Isolation and characterization of circulating tumor cells in patients with metastatic colorectal cancer. *Clin. Colorectal Cancer* 6(2), 125–132 (2006).

30 Hiraiwa K, Takeuchi H, Hasegawa H *et al.*: Clinical significance of circulating tumor cells in blood from patients with gastrointestinal cancers. *Ann. Surg. Oncol.* 15(11), 3092–3100 (2008).

31 Schwarzenbach H, Alix-Panabieres C, Muller I *et al.*: Cell-free tumor DNA in blood plasma as a marker for circulating tumor cells in prostate cancer. *Clin. Cancer Res.* 15(3), 1032–1038 (2009).

32 Lurje G, Schiesser M, Claudius A, Schneider PM: Circulating tumor cells in gastrointestinal malignancies: current techniques and clinical implications. *J. Oncol.* 2010, 392652 (2010).

33 Grimminger P, Vallböhmer D, Hoffmann A *et al.*: Quantitative analysis of survivin RNA expression in blood as a non-invasive predictor of response to neoadjuvant radiochemotherapy in esophageal cancer. *J. Surg. Oncol.* 100(6), 447–451 (2009).

34 Cao M, Yie SM, Wu SM *et al.*: Detection of survivin-expressing circulating cancer cells in the peripheral blood of patients with esophageal squamous cell carcinoma and its clinical significance. *Clin. Exp. Metastasis* 26(7), 751–758 (2009).

35 Yie SM, Lou B, Ye SR *et al.*: Detection of survivin-expressing circulating cancer cells (CCCs) in peripheral blood of patients with gastric and colorectal cancer reveals high risks of relapse. *Ann. Surg. Oncol.* 15(11), 3073–3082 (2008).

36 Yie SM, Lou B, Ye SR *et al.*: Clinical significance of detecting survivin-expressing circulating cancer cells in patients with non-small cell lung cancer. *Lung Cancer* 63(2), 284–290 (2009).

37 Yie SM, Luo B, Ye NY, Xie K, Ye SR: Detection of survivin-expressing circulating cancer cells in the peripheral blood of breast cancer patients by a RT-PCR ELISA. *Clin. Exp. Metastasis* 23(5–6), 279–289 (2006).

38 Warnecke-Eberz U, Hokita S, Xi H *et al.*: Overexpression of survivin mRNA is associated with a favorable prognosis following neoadjuvant radiochemotherapy in esophageal cancer. *Oncol. Rep.* 13(6), 1241–1246 (2005).

39 Klein CA: Cancer. The metastasis cascade. *Science* 321(5897), 1785–1787 (2008).

40 Ling X, Bernacki RJ, Brattain MG, Li F: Induction of survivin expression by taxol (paclitaxel) is an early event, which is independent of taxol-mediated G2/M arrest. *J. Biol. Chem.* 279(15), 15196–15203 (2004).

41 Kim JY, Chung JY, Lee SG *et al.*: Nuclear interaction of Smac/DIABLO with survivin at G2/M arrest prompts docetaxel-induced apoptosis in DU145 prostate cancer cells. *Biochem. Biophys. Res. Commun.* 350(4), 949–954 (2006).

42 Chandele A, Prasad V, Jagtap JC, Shukla R, Shastry PR: Upregulation of survivin in G2/M cells and inhibition of caspase 9 activity enhances resistance in staurosporine-induced apoptosis. *Neoplasia* 6(1), 29–40 (2004).

43 Beardmore VA, Ahonen LJ, Gorbsky GJ, Kallio MJ: Survivin dynamics increases at centromeres during G2/M phase transition and is regulated by microtubule-attachment and Aurora B kinase activity. *J. Cell Sci.* 117(Pt 18), 4033–4042 (2004).

44 Ghossein RA, Bhattacharya S, Rosai J: Molecular detection of micrometastases and circulating tumor cells in solid tumors. *Clin. Cancer Res.* 5(8), 1950–1960 (1999).

45 Ghossein RA, Coit D, Brennan M *et al.*: Prognostic significance of peripheral blood and bone marrow tyrosinase messenger RNA in malignant melanoma. *Clin. Cancer Res.* 4(2), 419–428 (1998).