

Review

Receptor-binding cancer antigen expressed on SiSo cells (RCAS1): a novel biomarker in the diagnosis and prognosis of human neoplasia

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Summary. The receptor-binding cancer antigen expressed on SiSo cells (RCAS1) is a novel tumor-associated antigen that induces cell-cycle arrest and/or apoptosis in RCAS1 receptor-bearing human cells. Current evidence has revealed enhanced RCAS1 expression in the tumor malignant stage of several organs, which may play a crucial role in tumor progression by enabling cancer cells to evade immune surveillance. In the last few years, tissue RCAS1 protein expression and circulating levels in biofluids have further been the focus of extensive research as a diagnostic and prognostic marker in several human malignancies. The present article aimed to review the available data so far concerning the clinical significance of RCAS1 in human neoplasia. Reviewing of English literature revealed that tissue RCAS1 expression was associated with important clinicopathological parameters for patients' management and prognosis, being considered as an informative biomarker in several types of human malignancy. In addition, the current evidence supported a crucial role for RCAS1 in tumor immune escape, which renders this receptor a promising target for future (gene) therapeutic approaches. However, the clinical application of circulating RCAS1 concentrations in biofluids as a marker in the management and prognosis of tumor malignancies needs to be further explored, since the data so far are still extremely limited.

Key words: RCAS1, Prognosis, Diagnosis, Cancer, Immune surveillance

Introduction

The receptor-binding cancer antigen expressed on SiSo cells (RCAS1) is a novel tumor-associated antigen that induces cell cycle arrest and/or apoptosis in RCAS1 receptor-bearing human cells (Nakashima et al., 1999). RCAS1 was initially recognized by the mouse monoclonal antibody, 22-1-1, which was raised by immunization of mice with the human uterine cervical adenocarcinoma cell line SiSo (Sonoda et al., 1996). It is a predominantly Golgi-localized membrane 40-Kd type II protein that forms homo-oligomers through its C-terminal coiled-coil structures, whereas it also exists in soluble form, probably by alternative splicing (Yamaguchi et al., 2005). Estrogen receptor-binding fragment associated antigen 9 (EBAG9), which is identical to RCAS1, was also identified as an estrogen-responsive gene product from a cDNA library of MCF-7 human breast cancer cell line (Watanabe et al., 1998).

Tumor infiltrating lymphocytes (TILs) are lymphocytes of the host immune system, such as normally activated T-cells, natural killer (NK) cells and non T- or non B- lymphocytes, which have been observed within tumor sites. TILs are deduced as a manifestation of the host immune response against tumor cells (Rosenberg, 1996). The presence of TILs has also been considered of prognostic importance, while inclusion of CD8+ T-cells into tumor cell nests seems to be a reliable prognostic indicator in several types of malignancies (Ropponen et al., 1997; Naito et al., 1998). However, the notion that infiltration of a tumor by TILs is always a prognostically positive phenomenon has changed in recent years, as research on extracellular matrix (ECM) remodeling and angiogenesis have identified numerous secreted factors which are common to both transformed cells and infiltrating leukocytes (Bodey et al., 2000).

Recent studies have revealed that RCAS1 can

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function as a ligand for a putative receptor present on various human cell lines, such as erythroid leukemia K562 cells and normal peripheral lymphocytes, T, B and NK cells, inhibiting cell growth and thus inducing apoptosis. Importantly, it was shown that RCAS1 was mainly produced by macrophages in bone marrow, exerting a crucial role in the control of erythropoiesis by modulating apoptosis in erythroid progenitor cells (Matsushima et al., 2001). Moreover, lipopolysaccharide (LPS)-stimulated macrophages induced cell death of erythroid progenitor cells through RCAS1 secretion (Suehiro et al., 2005). Substantial evidence further suggested that up-regulation of RCAS1 expression may play a crucial role in tumor progression by enabling cancer cells to evade immune surveillance (Nakashima et al., 1999; Akashi et al., 2003). More to the point, RCAS1 receptor expression was enhanced by activation of lymphocytes, and secreted RCAS1 in turn inhibited the *in vitro* growth of such activated cells, inducing their apoptotic cell death. Thus, tumor cells may evade immune surveillance by expressing RCAS1. In this context, Nakashima et al. also demonstrated that RCAS1 induced apoptosis in both cultured human lymphoma cell lines and normal peripheral lymphocytes, which express RCAS1 receptor (Nakashima et al., 1999). This apoptotic effect was supported to be mediated through induction of genes or caspase molecules, which strongly abrogated RCAS1-induced apoptosis, *in vitro* (Nakashima et al., 1999). RCAS1 was also shown to facilitate tumour cell invasion of connective tissue in

uterine cervical cancer via enhancement of invasive potency by induction of stromal tissue remodeling, as well as through evasion of antitumor immune surveillance by an apoptotic counter-attack mechanism against lymphocytes (Sonoda et al., 2005a). In this context, RCAS1 was shown to be secreted through ectodomain shedding, and its expression was related to alterations of ECM characteristics and to a reduced number of vimentin-positive tumor stromal cells, supporting evidence that RCAS1 may induce connective tissue remodeling (Sonoda et al., 2008). Moreover, RCAS1 may modulate surface expression of tumor-associated O-linked glycan structures, which are considered to participate in cell adhesion, invasion, and metastasis of cancer cells (Engelsberg et al., 2003). Additionally, knockdown of RCAS1 expression by RNA interference was also found to recover T-cell growth and proliferation. The suppression of RCAS1 expression effectively recovered T-cell proliferation, reduced apoptosis and partially reversed the T-cell function of IFN- γ secretion (Han et al., 2007). RCAS1 expression was also related to the abnormal expression of several proteins belonging to the cadherin family, which are considered to play a crucial role in the invasion and metastasis of colorectal carcinoma (Tsujitani et al., 2003). The existing biological functions of RCAS1 antigen are depicted in Figure 1.

In the last decade, tissue RCAS1 protein expression and circulating levels in biofluids have widely been studied in different malignant states as a biomarker in

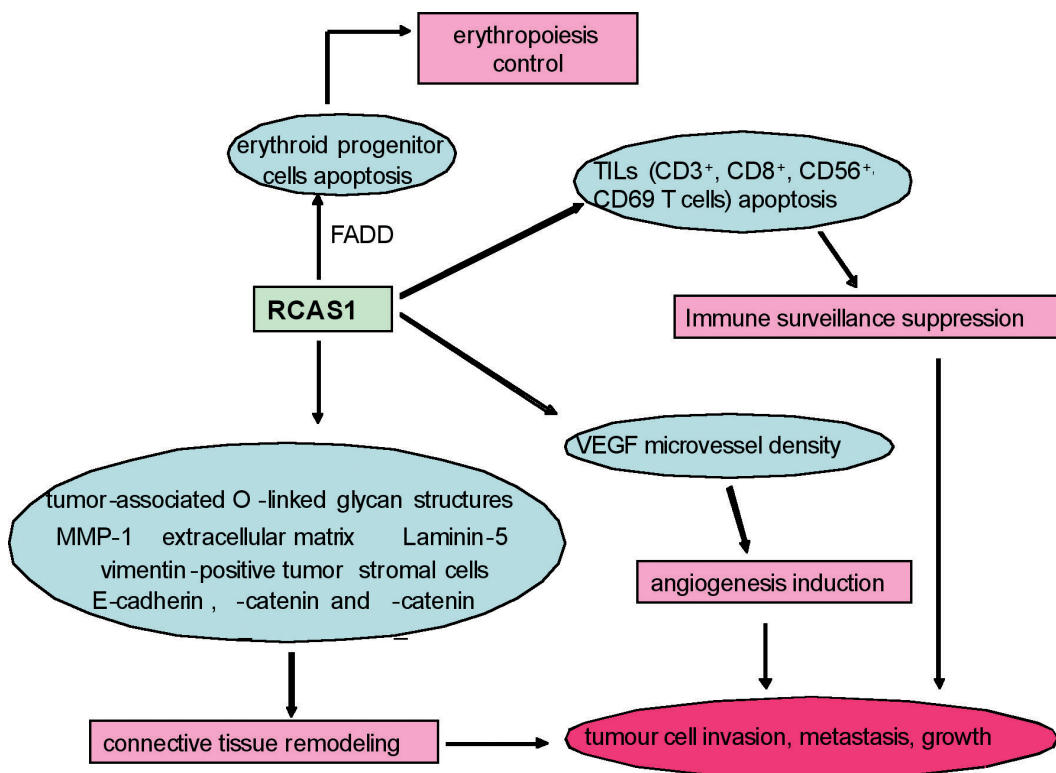


Fig. 1. The existing biological functions of RCAS1 antigen.

RCAS1 and human neoplasia

relation to clinicopathological parameters and patients' survival. The molecular mechanisms through which tumor cells may evade immune surveillance by expressing RCAS1 have also been the focus of extensive research efforts. In this aspect, we aimed to review the available data so far concerning the clinical significance of RCAS1 in human neoplasia. The crucial role of RCAS1 in tumor progression to enable cancer cells to evade immune surveillance is also discussed.

Tissue RCAS1 expression in human malignancy

Based on the evidence that RCAS1 plays a crucial role in tumor progression by enabling cancer cells to evade immune surveillance *in vitro*, several studies were conducted to evaluate the significance of tissue RCAS1 expression *in vivo*, in relation to clinicopathological characteristics, TILs apoptosis and patients' prognosis. The available data so far concerning the clinical significance of tissue RCAS1 expression in various human malignancies are discussed in the following paragraphs and are summarized in Tables 1 and 2.

Oral neoplasia

Neoplasia arising in the oral cavity is considered to progress from dysplasia to invasive carcinoma. In this

context, Toyoshima et al. showed that RCAS1 was weakly detected in the prickle and granular layers of the normal epithelia, but it was not found in any layer in cases of epithelial dysplasia (Toyoshima et al., 2006). In contrast, RCAS1 was strongly expressed in approximately 32% of squamous cell carcinoma (SCC) cases (Toyoshima et al., 2006). Accordingly, another study revealed a stepwise and significant increase in the expression of RCAS1 from normal oral mucosa through mild, moderate and severe oral epithelial dysplasia to SCC, supporting evidence that RCAS1 expression may be an early event in oral carcinogenesis (Tsai et al., 2008). An even higher incidence of RCAS1 positivity was reported by Fukuda et al. in 32 of 40 oral SCC cases (Fukuda et al., 2004). The same authors also detected significant RCAS1 transcript and protein levels in oral SCC cell lines, such as HSC-2, -3, -4, Ca9-22 and KB, while soluble RCAS1 determined by Western blotting was found to be secreted into culture supernatants of KB cells (Fukuda et al., 2004).

In 130 oral SCC cases studied by Toyoshima et al., RCAS1 expression was not significantly associated with patients' age and gender, tumor histopathological grade and stage, or tumor size and the mode of tumor invasion (Toyoshima et al., 2006). In contrast, the studies of Tsai et al., and Fukuda et al., which however were performed on a lower number of cases (84 and 40 oral SCCs,

Table 1. Associations of tissue RCAS1 expression with clinicopathological characteristics, TILs apoptosis and patients' prognosis in oral, esophageal, gastrointestinal, liver and gallbladder and pancreatic malignant tumors.

Type of Neoplasia	Sample Size	Age	Gender	Grade	Stage	pT	pN	pM	Type	TILs apoptosis	Survival	Ref
Oral	130	-	-	-	-	-				+	-	Toyoshima et al., 2006
	84	-	-		+	+	+				+	Tsai et al., 2008
	40				+					+		Fukuda et al., 2004
Esophageal	95	+	-	-	+	±	-	-			+	Nakakubo et al., 2002
	107	-	-	-	+	-	+			-	+	Tsujitani et al., 2007a
	114	-	-	-	+	-	+	-			+	Kato et al., 2005
	67					-	-				-	Ikeguchi et al., 2003a
Gastrointestinal												
Gastric	129	-	-	+		-	+			±	+	Fukuda et al., 2002
	54	-	-			+	+		+	+		Nakamura et al., 2004
Colorectal	58	-	-	-	+	-	+			+	+	Okada et al., 2003
	60	-	-	+	+	-	+	-				Leelawat et al., 2003
	105	-	-	-	+		+				+	Tsujitani et al., 2007b
	105				+			-			+	Tsujitani et al., 2003
GIMT	70										+	Naito et al., 2005
Liver and Gallbladder												
HCC	68	-	-	-	-	-						Noguchi et al., 2001
	60			-	-						-	Ikeguchi et al., 2003b
	143	-	-	+		-		-			-	Aoki et al., 2003
EBDC	60	-	-	-	-	-	-	-			+	Suzuoki et al., 2002
Gallbladder	46	-	-	-	+		+		-		+	Oshikiri et al., 2001
Pancreatic	76	-	-	+	-	±	-	-			-	Giaginis et al., 2008
	80	-	-	-	±	-	±				+	Hiraoka et al., 2002

negative tumors. Such a relationship was not found in patients with low dendritic cell infiltration (Tsujitani et al., 2007a). Thus, the authors suggested that the efficacy of RCAS1 to induce apoptosis may be restricted to lymphocytes activated by dendritic cells (Tsujitani et al., 2007a). This assumption was also supported by the data of Nakashima et al., who demonstrated that RCAS1 activity was only obvious against activated lymphocytes (Nakashima et al., 1999).

Most studies demonstrated that esophageal SCC patients with tumors presenting high RCAS1 expression were characterized by significantly lower overall survival times than those with low RCAS1 expression, while multivariate analysis rendered RCAS1 expression as an independent prognostic factor (Nakakubo et al., 2002; Kato et al., 2005; Tsujitani et al., 2007a). Accordingly, Ikeguchi et al. also showed that the 5-year survival rate of patients with RCAS1-positive tumors was lower than that of patients with -negative ones; however the differences were not statistically significant, which was ascribed to the small number of esophageal SCC cases analyzed (Ikeguchi et al., 2003a).

Thyroid neoplasia

RCAS1 expression was examined by immunohistochemistry in normal thyroid epithelium, follicular adenoma and carcinoma, as well as papillary and anaplastic carcinoma (Ito et al., 2003). Normal epithelium and follicular adenoma did not express, or only weakly expressed, RCAS1 protein. In thyroid carcinomas, RCAS1 overexpression was more frequently observed in anaplastic than papillary and follicular carcinomas. In follicular carcinoma, the widely invasive type more frequently overexpressed RCAS1 than the minimally invasive type. Furthermore, the incidences of RCAS1 overexpression increased with carcinoma dedifferentiation, supporting evidence that RCAS1 may contribute to the progression of thyroid carcinoma with high biological aggressiveness (Ito et al., 2003).

Gastric neoplasia

In gastric carcinomas, the incidence of RCAS1 expression was among the highest reported for other investigated types of neoplasia. RCAS1 was also expressed in non-cancerous gastric epithelial cells, including gastric adenomas, ulcers and normal epithelia (Kubokawa et al., 2001; Fukuda et al., 2002; Nakamura et al., 2004). Nevertheless, a striking difference in the pattern of RCAS1 expression was found between benign and malignant cells. In cases of normal gastric mucosa, such as gastric ulcers and adenomas, RCAS1 was localized in the perinuclear region of the mucosal epithelial cells, defined as PN pattern, whereas in most cases of gastric carcinomas it was detected diffusely in both the cytoplasm and cell membranes of the tumor cells, defined as DC pattern (Kubokawa et al., 2001;

Nakamura et al., 2004). In addition, RCAS1 mRNA levels, determined by a semi-quantitative RT-PCR analysis, were found to be significantly higher than those in non-neoplastic ones (Kubokawa et al., 2001). The PN pattern of RCAS1 expression was significantly more frequently observed in well differentiated adenocarcinomas than in moderately differentiated ones, while the DC pattern was more frequently recognized in carcinomas which invaded beyond the submucosa compared to intramucosal carcinomas (Kubokawa et al., 2001).

In the study of Fukuda et al., which was performed on 129 T3 gastric carcinoma cases, RCAS1 expression was significantly correlated with tumor histopathological grade and lymph node metastasis (Fukuda et al., 2002). In this context, Nakamura et al. documented a significant association between RCAS1 expression pattern and tumor histopathological type, as diffuse type carcinomas more frequently presented DC pattern (Nakamura et al., 2004). In addition, the DC pattern of RCAS1 expression was more frequently detected in cases with higher tumor size, invasion beyond the submucosa and regional lymph node metastasis compared with those presenting PN pattern. This difference in the RCAS1 distribution was ascribed to differences of function, indicating a death ligand for immune cells, malignant transformation or progression (Nakamura et al., 2004). It should be noted that both studies revealed that the rate of TILs apoptosis was significantly higher in gastric carcinoma patients with RCAS1-positive than -negative tumors (Fukuda et al., 2002; Nakamura et al., 2004). Thus, it was assumed that tumors may escape from the immune system by expressing RCAS1, which in turn may induce apoptosis in RCAS1 receptor-positive T-cells.

As regards the prognostic value of RCAS1, Fukuda et al. showed that the patients with high RCAS1 expression presented significantly shorter survival times than those with low RCAS1 expression (Fukuda et al., 2002). In this study, RCAS1, tumor size and lymph node metastasis were identified as independent prognostic factors in multivariate analysis. Moreover, RCAS1 expression was significantly associated with poor prognosis in the subgroup of patients with a high rate of TIL apoptosis. In contrast, there was no significant difference between RCAS1 expression levels in the subgroup of patients with a low rate of TIL apoptosis (Fukuda et al., 2002).

Colorectal neoplasia

A significant number of studies documented that RCAS1 was strongly expressed in colorectal carcinoma, presenting DC pattern, whereas weak RCAS1 expression was noted in the normal mucosa. In this type of neoplasia, all studies reported significant association between RCAS1 expression and tumor histopathological stage, as well as lymph node metastasis, whereas no significant correlations with patients' age and gender, tumor size and organ metastasis were noted (Leelewat et

al., 2003; Okada et al., 2003; Tsujitani et al., 2003, 2007b). The study of Leelawat et al., conducted on 60 colorectal carcinomas, further reported a significant association between immunohistochemical RCAS1 expression and tumor histopathological grade, which is in contrast to the studies of Okada et al. and Tsujitani et al. (Leelawat et al., 2003; Okada et al., 2003; Tsujitani et al., 2007b). In a previous study by Tsujitani et al. it was documented that RCAS1 expression was associated with the abnormal expression of E-cadherin, α -catenin and β -catenin. Interestingly, cadherin molecules are considered to play crucial role in the invasion and metastasis of colorectal carcinoma (Tsujitani et al., 2003). In this context, E-cadherin, the major cadherin molecule expressed by epithelial cells, has been considered as an invasion-suppressor, being extensively studied in both experimental and human cancers (Tsujitani et al., 2007b).

Only one study investigated the relationship of RCAS1 expression and the frequency of apoptosis among TILs, in vivo (Okada et al., 2003). In this context, the proportion of apoptotic TILs was significantly higher in RCAS1-positive than in -negative colorectal carcinomas. These findings suggested that tumor-derived RCAS1 may induce TIL apoptosis through RCAS1-receptor, leading to immune cell depletion in human colorectal carcinomas (Okada et al., 2003). However, the previous data where the prognostic value of the presence of TILs, including CD8+ T-cells, depends on RCAS1 expression, was opposed to that provided by Oshikiri et al. which indicated the independence of Tn glycan antigen and CD8+ T-cells as prognostic indicators in colorectal cancer (Oshikiri et al., 2006).

Survival analysis showed that colorectal carcinoma patients with RCAS1-positive tumors presented a significantly poorer prognosis than RCAS1 negative ones, while multivariate analysis indicated that RCAS1 positivity was an independent negative predictor for patients' survival (Okada et al., 2003; Tsujitani et al., 2003, 2007b). Tsujitani et al. further revealed that reduced or abnormal expression of E-cadherin was an independent negative prognostic factor in RCAS1-positive patients with expression, but not in -negative ones, reinforcing the assumption that E-cadherin and RCAS1 are interrelated and may be critical for the mechanism of metastasis and recurrence in human colorectal cancer (Tsujitani et al., 2007b).

Gastrointestinal (GI) tract neoplasia

Gastrointestinal mesenchymal tumors (GIMTs) are the most common mesenchymal tumors of the gastrointestinal tract. In this context, univariate analysis for a recurrence-free prognosis demonstrated that RCAS1 expression was correlated with a significantly higher potential of recurrence (Naito et al., 2005). On multivariate analysis, both tumor size and RCAS1 expression proved to be independently and inversely

correlated with recurrence-free survival, suggesting that RCAS1 expression may be related with recurrence not only in carcinomas, but also in mesenchymal tumors (Naito et al., 2005).

Liver and gallbladder neoplasia

RCAS1 was expressed in 26.5% of hepatocellular carcinoma (HCC) cases, while the percentage of vascular and/or bile ductal invasion was significantly much higher in RCAS1-positive than in -negative patients' group (Noguchi et al., 2001). These findings suggested that RCAS1 expression was associated with increased tumor invasiveness. Since in vitro evidence indicated that RCAS1 inhibited the growth of receptor-expressing immune cells and induced apoptotic cell death (Nakashima et al., 1999), this RCAS1 effect to evade immune surveillance may, at least in part, contribute to tumor invasiveness (Noguchi et al., 2001). However, it remains to be clarified whether RCAS1 is the main apoptosis-inducer against immune cells, as apoptosis induction in activated lymphocytes has been reported for other molecules, such as CD95 ligand and DF3/MUC1, while HCC cells frequently express CD95 ligand (Strand et al., 1996; Walker et al., 1998).

As regards the diagnostic value of RCAS1 in HCC, two studies showed no significant association between RCAS1 protein expression and tumor histopathological grade and stage, as well as tumor size (Noguchi et al., 2001; Ikeguchi et al., 2003b). However, in another study performed on a higher number of HCC cases, enhanced RCAS1 expression was significantly associated with reduced degree of differentiation and cell proliferation, reflected by Ki-67 labeling index (Aoki et al., 2003). In this study, there was also a constant low level of RCAS1 expression in non-tumoral liver tissues, with a regular cytoplasmic distribution (Aoki et al., 2003). This finding of RCAS1 expression in non-tumoral hepatocytes contradicts the results provided by Noguchi et al. (Noguchi et al., 2001). Interestingly, tumors that showed a 'nodule-in-nodule' appearance displayed a variable degree of RCAS1 expression that depended on the degree of differentiation within the tumor, which further supports the link between the level of RCAS1 expression and tumor progression in HCC (Aoki et al., 2003).

Concerning the prognostic value of RCAS1 in HCC, both Aoki et al. and Ikeguchi et al. did not reveal significant relationship between RCAS1 expression and disease-free survival (Aoki et al., 2003; Ikeguchi et al., 2003b). On the other hand, Ikeguchi et al. further showed that in the case of primary tumors after transcatheter arterial embolization (TAE) treatment, reduced RCAS1 expression was a poor independent prognostic factor (Ikeguchi et al., 2004). Although these data supported that RCAS1 expression may be a good marker for the effectiveness of TAE treatment in patients who develop new tumors in the residual livers, they were opposed to the majority of studies presented in this review, which rendered high RCAS1 expression as a

poor prognostic factor. This discrepancy may be ascribed to the low number of cases ($n=39$) used by Ikeguchi et al. for survival analysis (Ikeguchi et al., 2004). In this study, it was also revealed that the density of CD8+ T-cells in tumors was significantly lower than in non-cancerous hepatic lobules and in relation to stage progression (Ikeguchi et al., 2004). The density of CD8+ T-cells in tumors positively correlated with the occurrence of tumor cell apoptosis, but did not correlate with RCAS1 protein expression. Thus, it was assumed that CD8+ TILs may play a role in the occurrence of tumor cell apoptosis in HCC, but CD8+ TILs may not be controlled by RCAS1 in this type of malignancy (Ikeguchi et al., 2004).

Furthermore, RCAS1 was expressed in 52 of 60 extrahepatic bile duct carcinoma cases (Suzuoki et al., 2002). Normal bile ductal epithelium did not present RCAS1 positivity; however, RCAS1 was very frequently expressed in stage I disease (Suzuoki et al., 2002). No correlations between RCAS1 expression and patients' age and gender, tumor histopathological grade and stage, tumor size, lymph node and organ metastasis status, as well as lymphatic, venous and perineural invasion were noted, although high RCAS1 expression correlated with poor prognosis (Suzuoki et al., 2002). In multivariate analysis, high RCAS1 expression proved an independent negative predictor for patients' survival, supporting evidence that the capacity of tumor cells to escape immune surveillance conferred by the expression of RCAS1 may play an important role in determining the outcome of patients with extrahepatic bile duct carcinoma (Suzuoki et al., 2002).

High RCAS1 expression was also found in 32 of the 46 gallbladder cancer specimens (70%), but not in cases of cholecystitis, adenomyomatosis and adenomas (Oshikiri et al., 2001). RCAS1 immunoreactivity was associated with the depth of tumor invasion, lymph node metastasis, as well as lymphatic, vascular and perineural involvement and TNM stage (Oshikiri et al., 2001). Thus, RCAS1 expression was supported to be a relatively late event in gallbladder carcinogenesis, possibly promoting tumor progression. Gallbladder cells expressing RCAS1 may escape immune surveillance and survive in lymphatic vessels, lymph nodes and the ECM. Multivariate analysis also identified RCAS1 positivity as an independent negative predictor for patients' survival (Oshikiri et al., 2001).

Pancreatic neoplasia

In a recent study of our group, RCAS1 positivity was noted in 86% (65/76) of the examined cases of pancreatic adenocarcinoma (Giaginis et al., 2008). This incidence of pancreatic adenocarcinoma RCAS1 positivity is among the highest already reported for other types of carcinomas, but not the highest among the incidences reported for this specific type of malignancy. In fact, both Hiraoka et al. (Hiraoka et al., 2002) and Akashi et al. (2003) have reported an even higher

incidence of RCAS1 positivity, 96% (77/80), 100% (20/20), respectively. It should be noted that the intensity of RCAS1 staining in our study was classified as moderate and intense at a percentage of 88% of the positive cases, which is almost equal to that reported by Hiraoka et al. (87%) (Hiraoka et al., 2002).

Taken into consideration these findings along with our results, the assumption could be supported that pancreatic adenocarcinoma bears a high probability for RCAS1 expression. On the other hand, it is well-established that when the diagnosis is made on the basis of biopsy findings, chronic pancreatitis can mimic adenocarcinoma, since it might present significant epithelial atypia and fibrosis (Evans et al., 1997). In this context, RCAS1 expression is considered to be a relatively frequent finding in chronic pancreatitis cases. Indeed, an incidence of 40% (2/5) of chronic pancreatitis cases has been reported by Akashi et al. (2003); however no precise conclusions should be drawn due to the small number of the examined cases.

Concerning the diagnostic value of RCAS1 expression, our previous study showed a significant correlation between RCAS1 positivity and pancreatic adenocarcinoma differentiation, as well as a trend of correlation with tumor size (Giaginis et al., 2008). Moreover, both RCAS1 staining intensity and overexpression presented a trend of correlation with tumor histopathological grade (Giaginis et al., 2008). In contrast, Hiraoka et al., using 80 pancreatic adenocarcinoma cases, reported borderline associations between RCAS1 expression and lymph node metastasis, as well as tumor histopathological stage (Hiraoka et al., 2002). In addition, the survival of patients with high RCAS1 expression was significantly shorter than that of those with low expression, and multivariate analysis rendered high RCAS1 expression as an independent prognostic factor (Hiraoka et al., 2002). In contrast, no correlation was noted between RCAS1 positivity, staining intensity or overexpression and the prediction of patients' survival in our study (Giaginis et al., 2008). This discrepancy may be ascribed to the criteria for RCAS1 immunoreactivity definition and the different cutoff points of the percentage of RCAS1 positive malignant cells that were set.

Lung neoplasia

In lung carcinomas, RCAS1 was strongly expressed, presenting DC pattern. RCAS1 was also detected in normal ciliated columnar epithelial cells, especially in goblet cells of the bronchi and the bronchioles (Iwasaki et al., 2000; Izumi et al., 2001; Oizumi et al., 2002). In two studies performed on non-small cell lung carcinoma (NSCLC) cases, RCAS1 expression was significantly associated with the tumor histopathological grade and stage (Iwasaki et al., 2000; Izumi et al., 2001). In fact, RCAS1 expression in NSCLC cases with high tumor size or advanced disease stage was significantly or marginally significantly higher compared to cases with

small tumor size or non-advanced disease stage (Iwasaki et al., 2000). Moreover, RCAS1 was more frequently expressed in poorly differentiated adenocarcinomas than in moderately or well-differentiated ones (Iwasaki et al., 2000). On the other hand, Oizumi et al. reported no significant associations of RCAS1 expression with tumor histopathological grade and stage; however, this study was performed on lung adenocarcinomas and did not include SCC cases (Oizumi et al., 2002). Concerning the expression of RCAS1 among the different histopathological types, Iwasaki et al. showed that it was significantly higher in SCCs than in adenocarcinomas (Iwasaki et al., 2000), whereas Izumi et al. did not find any significant relationship between RCAS1 expression and tumor histopathological type (Izumi et al., 2001).

In NSCLC cases with increased RCAS1 expression, the apoptotic index of TILs was significantly higher than those with low expression, suggesting that RCAS1 expression on tumor cells may induce apoptosis in TILs and contribute to tumor escape from the immune system (Iwasaki et al., 2000).

Concerning the prognostic value of RCAS1 expression, NSCLC patients with tumors presenting high RCAS1 expression were characterized by significantly lower overall survival than those with low RCAS1 expression (Iwasaki et al., 2000; Izumi et al., 2001). Multivariate analysis also rendered RCAS1 expression as an independent prognostic factor (Iwasaki et al., 2000; Izumi et al., 2001). Accordingly, the study of Oizumi et al., which was performed on 70 lung adenocarcinomas and did not include other histopathological types of lung carcinomas, showed that RCAS1 expression was a significant and independent prognostic factor (Oizumi et al., 2002). These data suggested that lung carcinomas expressing RCAS1 may be invasive and progressive, and such characteristics may, at least in part, lead to poor prognosis. Therefore, it was speculated that patients with lung carcinoma and strong RCAS1 expression in a preoperative biopsy specimen may require either an exhaustive search for metastases or a decision for neoadjuvant therapy (Izumi et al., 2001). An immunohistochemical analysis performed by tissue microarrays (TMA) on 35 archival stage II NSCLC tissues further confirmed the significant association between RCAS1 expression and histopathological stage, as well as tumor differentiation and poor prognosis (Kohri et al., 2006). However, in a more recent study restricted to stage IB-IIIa NSCLC cases, no significant relationship between RCAS1 expression and overall or disease-free survival was noted (Timotheadou et al., 2007).

In another study, Hiraki et al. evaluated the involvement of RCAS1 on the survival of patients with malignant mesothelioma (Hiraki et al., 2005). In 38 patients with pleural malignant mesothelioma, a positive rate of 89.5% was found for RCAS1 expression. In fact, the positive rate was 90.9% in biphasic, 78.6% in epithelioid type, and 100% in sarcomatoid types. No significant association was observed between RCAS1

expression and patients' gender and age, histopathological type or clinical stage (Hiraki et al., 2005). The survival of malignant mesothelioma patients with RCAS1-positive tumors was significantly increased compared to those with -negative ones, while multivariate analysis revealed that RCAS1 positivity exerted a significantly positive effect on survival (Hiraki et al., 2005). However, these data are in contrast to the majority of studies, which identified high RCAS1 expression as a poor prognostic factor in lung cancer patients and may be ascribed to the low number of cases (n=38) used by Hiraki et al. for survival analysis.

Breast neoplasia

In invasive breast ductal carcinoma, RCAS1 immunoreactivity was detected by immunohistochemistry in the entire surface and cytoplasm of tumor cells, whereas in non-neoplastic mammary glands RCAS1 was weakly or not expressed on the luminal surface of epithelial cells (Suzuki et al., 2001; Rousseau et al., 2002). In addition, significantly higher RCAS1 expression was noticed in tumor than stroma sites in breast cancer patients with lymph node metastases, whereas no such difference was observed in non metastatic cases (Suzuki et al., 2001; Rousseau et al., 2002). These findings were ascribed to alterations in the number and activity of immune cells, supporting evidence that RCAS1 expression in breast cancer healthy stroma may be essential for the coexistence of cytotoxic immune cells and normal epithelial ones (Suzuki et al., 2001; Rousseau et al., 2002). These data also reinforce the assumption that the loss of the ability to compensate the growing cytotoxic immune response in the environment might participate in the development of tumor spread (Popiela et al., 2006). Rousseau et al. also revealed that RCAS1 expression presented only cytoplasmic distribution pattern in cancer cells obtained from human ductal breast cancer specimens (Rousseau et al., 2002). As also observed in human breast cancer patients, RCAS1 protein was strongly expressed in the cytoplasm of MCF7, MDA-MB-231 and BT-474 cell lines (Rousseau et al., 2002).

Furthermore, the current studies did not reveal significant associations between RCAS1 expression and patients' age, tumor size, lymph node metastasis and tumor histopathological stage in breast cancer (Suzuki et al., 2001; Rousseau et al., 2002). In the study of Rousseau et al., conducted on 37 histological specimens, and in another performed by our group on 79 cytological specimens, RCAS1 protein expression was significantly increased with tumor histopathological grade (Rousseau et al., 2002; Theoharis et al., 2006). However, no such relationship was reported by Suzuki et al. (2001). We further revealed a significant association of RCAS1 expression with the presence of organ metastasis and tumor proliferative capacity, reflected by Ki-67 labeling index (Theoharis et al., 2006). Interestingly, Suzuki et al. further showed that RCAS1 immunoreactivity was

significantly associated with estrogen receptor (ER)- α expression and inversely associated with the degree of intra-tumoral infiltration of mononuclear cells or CD3⁺ T-lymphocytes (Suzuki et al., 2001). Although this association did not reach statistical significance, a similar tendency between RCAS1 and ER β was also noted (Suzuki et al., 2001). Thus, it was supported that RCAS1 may be produced through ER in tumor cells, inhibiting the intra-tumoral infiltration of T-lymphocytes in the context of a possible endocrine-immune interaction in human breast carcinoma (Suzuki et al., 2001). On the other hand, both Theoharis et al. and Rousseau et al. documented that RCAS1 expression did not significantly correlate with ER and progesterone receptor (PR) expression pattern, indicating that RCAS1 protein synthesis may be regulated by additional factors other than estrogens in breast carcinoma tissues (Rousseau et al., 2002; Theoharis et al., 2006).

Ovarian neoplasia

RCAS1 was highly expressed in ovarian cancer tissues, but not in normal ovaries (Sonoda et al., 1996; Kawano et al., 2005). Accordingly, Chatterjee et al. showed that RCAS1 expression, assessed by immunohistochemistry, was highly expressed in 22 of 30 stage I tumors, 26 of 39 late-stage tumors, but only 1 of 20 normal ovary tissues (Chatterjee et al., 2006). In 90 epithelial ovarian cancer cases, Akahira et al. reported that RCAS1 expression was significantly higher in patients with serous type tumors and advanced stage disease, whereas there were no such relationships between RCAS1 expression and patients' age, tumor histopathological grade and residual tumors (Akahira et al., 2004). A highly significant correlation between RCAS1 and ER α immunoreactivity was noted, whereas there was no significant relationship between RCAS1 immunoreactivity and patients' overall survival. Moreover, all positive cases for ER α mRNA were also positive for RCAS1 mRNA, suggesting that the upregulation of RCAS1 may be under estrogen control in ovarian epithelial carcinoma (Akahira et al., 2004).

Endometrial neoplasia

Immunohistochemical studies in endometrium tissue specimens revealed a gradually increased incidence for RCAS1 expression from normal uterine endometrium to hyperplasia and endometrial carcinoma (Sonoda et al., 2000; Zhou et al., 2008). PC pattern was identified in normal, simple and complex hyperplastic endometrium, whereas DC pattern was predominantly found in the endometrium in cases of malignancy (Sonoda et al., 2000; Zhou et al., 2008). RCAS1 expression was significantly higher in cases of adenocarcinoma than in normal and hyperplastic endometrium (Sonoda et al., 2000; Zhou et al., 2008). In addition, RCAS1 was expressed in endometrioid adenocarcinoma, but not in any of the serous or clear cell adenocarcinoma cases

(Sonoda et al., 2000; Zhou et al., 2008). Endometrial adenocarcinoma was also characterized by significantly increased RCAS1 expression compared to endometriosis (Wicherek et al., 2005).

Sonoda et al. also showed that RCAS1 was more frequently expressed in grade 3 than in grade 1 or 2 endometrial carcinomas (Sonoda et al., 2000). In contrast, Zhou et al. did not find a significant relationship between RCAS1 expression and tumor histopathological grade, which may be ascribed to the considerably lower number of the examined cases (Zhou et al., 2008). Zhou et al. further reported that high RCAS1 expression was significantly related to myometrial and vascular invasion in endometrial adenocarcinoma cases (Zhou et al., 2008). Moreover, RCAS1 expression was significantly higher in ER α -positive than -negative cases. Hence, both the expression and distribution of RCAS1 may be involved in the malignant transformation of endometrium, and RCAS1 co-expression with ER α may be associated with tumor growth and metastasis in this type of carcinoma (Zhou et al., 2008).

Cervical neoplasia

In uterine cervical carcinoma, Sonoda et al. reported strong RCAS1 expression in both SCC and adenocarcinoma (Sonoda et al., 2005a). RCAS1 expression in lymph nodes was significantly higher than that of primary lesions in patients with cervical cancer and was associated with a less favorable prognosis. In addition, RCAS1 expression was significantly associated with the number of apoptotic lymphocytes in primary lesions of uterine cervical cancer and metastatic lymph nodes (Sonoda et al., 2005a). It should be noted that T-lymphocytes are an essential feature of the host defense system against the neoplastic process, and several apoptosis-inducing molecules, such as FasL, TNF- α , and RCAS1, have been implicated in suppression of T-lymphocyte function. However, no significant association in the case of FasL and TNF- α was noted, suggesting that RCAS1, but not FasL and TNF- α , may be produced by tumor cells to induce suppression of immune surveillance in the microenvironment of cervical cancer (Sonoda et al., 2005a).

The occurrence and degree of RCAS1 expression increased with the depth of invasion in cervical neoplasia, ranging from precancerous state to invasive cancer (Sonoda et al., 2005b). In the subgroup of patients with invasive carcinoma, RCAS1 overexpression was significantly associated with the invasion of lymph-vascular space, lymph node metastasis and tumor burden (Sonoda et al., 2005b). Overall survival rates of patients with tumors presenting high RCAS1 expression were significantly shorter than those with low RCAS1 expression. To estimate the effect of RCAS1 expression on remodeling of tumor stromal tissue, Sonoda et al. further measured the number of stromal cells with vimentin expression (Sonoda et al.,

2005b). In this regard, the number of vimentin-positive cells significantly decreased in patients with higher RCAS1 expression compared to those with lower RCAS1 expression. Moreover, significant associations were found between RCAS1 and other molecules involved in tumor cell invasion, such as MMP-1 and laminin-5. Thus, it was speculated that RCAS1 may contribute to the acquisition of malignant uterine cervical phenotypic characteristics, including invasion, metastasis, and tumor growth via connective tissue remodelling (Sonoda et al., 2005b).

Furthermore, it was shown that RCAS1 promoted angiogenesis and accelerated *in vivo* tumor growth in nude mice in which the immune system was defective (Sonoda et al., 2007a). These data indicated that RCAS1 may influence tumor-stromal interaction and enhance angiogenesis, which are critical for tumor growth, *in vivo*. In 123 archival uterine cervical carcinoma tissue samples, RCAS1 expression was also significantly associated with VEGF expression and microvessel density, further supporting that RCAS1 was associated with angiogenesis in uterine cervical cancer (Sonoda et al., 2007a).

Strong RCAS1 expression was also reported in normal female genital organs from 66 patients (Kawano et al., 2005). In uterine cervical glands, RCAS1 expression was detected in 93% of the examined cases, being localized mainly in the superficial area, while strong RCAS1 expression was also noted in all samples in the vicinity of areas with squamous metaplasia. 84% of the examined cases stained positive for RCAS1 in the uterine cervical squamous epithelium, while another 87% in the uterine corpus, being mainly expressed in the endometrial glands of the basal layer. There was also a significant positive correlation between patients' age and RCAS1 expression, but no significant difference between RCAS1 expression and endometrial status, or cell proliferation and apoptosis (Kawano et al., 2005).

Prostatic neoplasia

Immunohistochemical analysis revealed strong and diffuse RCAS1 immunoreactivity in 44 of 81 (54%) prostatic cancer specimens (Takahashi et al., 2003). RCAS1 expression was significantly associated with advanced tumor histopathological stage and high Gleason score (Takahashi et al., 2003). In addition, RCAS1 was more frequently expressed at sites of capsular penetration and lymph node metastasis compared to intracapsular primary tumors. Patients with RCAS1-positive prostatic tumors were characterized by significantly worse Prostate Specific Antigen (PSA) failure-free survival than those with -negative tumors (Takahashi et al., 2003). However, in multivariate analysis, RCAS1 expression was not an independent prognostic factor. A trend was also found that CD3⁺ cells infiltrated less into the margin of RCAS1-positive than -negative tumors, suggesting that prostatic cancers expressing RCAS1 may evade immune surveillance and

subsequently progress to metastatic disease (Takahashi et al., 2003).

Neurological neoplasia

RCAS1 expression was detected as DC pattern in gliomas, but not in normal brain tissues (Nakabayashi et al., 2007). Significant associations of enhanced RCAS1 expression with increased tumor histopathological grade and stage, as well as cell proliferation, reflected by Ki-67 labelling index, were noted. Patients with tumors presenting high RCAS1 expression were characterized by significantly shorter disease-free survival than those with low RCAS1 expression (Nakabayashi et al., 2007). Reduced infiltration and increased apoptosis of TILs was found in the RCAS1-positive regions of the same sections, suggesting that RCAS1 expression in gliomas may play critical roles in both tumor progression and immune escape (Nakabayashi et al., 2007). Umeoka et al. also showed that RCAS1 was expressed in 48% of pituitary adenomas, but not in normal pituitary glands. RCAS1 expression was not associated with the type of pituitary adenomas, tumor size and invasiveness; however, a significant relationship between RCAS1 and Ki-67 positivity was found (Umeoka et al., 2001).

Skin neoplasia

An immunohistochemical study performed on various skin tumors revealed a high incidence of RCAS1 positivity (74%) in SCC cases (Takahashi et al., 2001). RCAS1 was not detected in normal human epidermis or in Bowen's disease, whereas low incidence of RCAS1 positivity was noted in seborrheic keratosis (5%), actinic keratosis (8%), keratoacanthomas (13%) and basal cell carcinomas (14%) tissue samples (Takahashi et al., 2001). In SCCs, RCAS1 expression was significantly associated with tumor histopathological stage. In addition, RCAS1 was found to be highly expressed in extramammary Paget's disease. Fifty nine of 63 extramammary Paget's disease samples (94%) were positive for RCAS1, while 58 (92%) showed co-expression of RCAS1 and CEA. Two of 24 melanoma cases (8%) also expressed RCAS1, whereas none of 20 nevus pigmentosus showed positive staining (Takahashi et al., 2001).

Haematological malignancy

In lymph node specimens obtained from patients with adult T-cell leukemia/lymphoma (ATLL), positive RCAS1 staining was detected in 15 (75%) of 20 cases and in all cases of patients with short survival times (Muta et al., 2004). In B-cell lymphomas, positive RCAS1 staining was detected in only 1 of 8 (13%) cases (Muta et al., 2004). These findings indicated that RCAS1 expression may be associated with the evasion from immune surveillance of cells infected with human T-lymphotropic virus type I, resulting in the

development of overt leukemia/lymphoma (Muta et al., 2004). In this respect, it should be emphasized that RCAS1 was reported to be produced by macrophages in hematopoietic tissue and may have a crucial role in the control of erythropoiesis by modulating apoptosis of erythroid progenitor cells via a Fas-independent mechanism (Matsushima et al., 2001). RCAS1 was shown to activate caspase-8, inducing apoptosis of erythroid progenitors via FADD, which is a molecular upstream of caspase-8 (Matsushima et al., 2001).

In this aspect, RCAS1 was also found to be expressed on macrophages, playing an important role in the induction of activated T-cell apoptosis in cases of histiocytic necrotizing lymphadenitis (HNL), which was characterized by necrotic lesions consisting of T-cells undergoing apoptosis and macrophages in proliferation (Abe et al., 2003). In fact, RCAS1 expression was analyzed by immunohistochemistry in 9 cases of HNL, while 9 cases of reactive lymphadenitis were used as control (Abe et al., 2003). The ratio of RCAS1/CD68⁺ positive cells was significantly higher in patients with HNL than in those with reactive lymphadenitis (Abe et al., 2003). It was also suggested that macrophages may negatively regulate erythropoiesis, at least in part, through the production of RCAS1 molecules, and this fact may play a crucial role in the pathogenesis of the anemia of chronic disease and inflammation (Suehiro et al., 2005). In particular, when macrophages were stimulated with LPS, the expression of RCAS1 was remarkably enhanced, while LPS-stimulated macrophages induced cell death of erythroid progenitor cells through RCAS1 production (Suehiro et al., 2005).

Circulating RCAS1 levels in biofluids

A significantly lower number of studies were conducted to evaluate the circulating RCAS1 levels in biofluids as a biomarker for the diagnosis and prognosis of human neoplasia. The available data so far concerning the clinical significance of circulating RCAS1 levels in biofluids are discussed in the following paragraphs and are summarized in Table 3.

Gastrointestinal neoplasia

Mean serum RCAS1 levels were found to be significantly higher in patients with GI tract tumors

compared with the control group (Coban et al., 2006). In fact, among the GI tract tumors, RCAS1 presented lowest and highest sensitivity for esophagus and colon cancer diagnosis, respectively (Coban et al., 2006).

Moreover, serum RCAS1 exhibited a higher sensitivity for malignancy, except in the colon, and lower specificity in all groups compared with the known tumor marker carcinoembryonic antigen (CEA). In comparison with carbohydrate antigen (CA) 19-9, serum RCAS1 also proved more sensitive, but less specific for all gastrointestinal cancer groups tested (Coban et al., 2006). In addition, serum RCAS1 levels were significantly higher in cases with lymph node involvement compared with lymph node-negative cases; however, there was no difference between cases with and without serosal involvement, vascular invasion and distant metastasis (Coban et al., 2006).

Colorectal neoplasia

Leelawat et al. revealed that serum RCAS1 levels were upregulated in advanced stages of colorectal cancer (Leelawat et al., 2003). In fact, positive serum RCAS1 concentrations were found in 10 of 18 patients with stage II disease and 12 of 32 with stage III and IV, but not in patients with stage I disease. On the other hand, in this study, serum RCAS1 concentration in patients with colorectal cancer was not significantly different from normal controls (Leelawat et al., 2003).

Liver neoplasia

Serum RCAS1 levels were shown to exceed the normal limit in a high percentage (74%) of cholangiocellular carcinoma patients (Watanabe et al., 2003). RCAS1 positivity rate was higher than those of CA19-9 and CEA; however, the differences did not reach statistical significance (Watanabe et al., 2003). Serum RCAS1 was also positive in many cases that were negative for CA19-9. Surgical resection of cholangiocellular carcinoma reduced RCAS1 levels within the normal range. Thus, in cholangiocellular carcinoma, serum RCAS1 concentration was considered as a tumor marker of complementary value, and therefore measuring serum RCAS1 in addition to CA19-9 and CEA may contribute to the diagnostic accuracy, being useful for estimating tumor progression or

Table 3. Associations of circulating RCAS1 levels in biofluids with clinicopathological characteristics and patients' prognosis.

Type of Neoplasia	Sample Size	Sample type	Age	Gender	Grade	Stage	pT	pN	pM	Type	Survival	Ref
Gastrointestinal	82	Serum					-	+	-	+		Coban et al., 2006
Lung	45	Pleural fluid	-	-					-	-	+	Aoe et al., 2006
Ovarian	54	Serum			-	-				+		Sonoda et al., 2007b
Endometrial	50	Serum	-			-		-		-		Sonoda et al., 2006
Cervical	63	Serum	-			±		-		+		Sonoda et al., 2006

therapeutic effect (Watanabe et al., 2003).

Pancreatic neoplasia

Akashi et al. supported evidence that serum RCAS1 exhibited similar specificity and higher sensitivity than CA19-9, a highly sensitive marker for pancreatic cancer (Akashi et al., 2003). In addition, serum RCAS1 concentrations in patients with ductal adenocarcinoma of the pancreas were significantly higher than those in patients with other inflammatory pancreatic diseases, such as chronic, acute and autoimmune pancreatitis. RCAS1 concentrations in patients with intraductal papillary-mucinous adenoma of the pancreas were also significantly higher than those in patients with chronic and autoimmune pancreatitis (Akashi et al., 2003). In another study, the serum RCAS1 levels were also shown to exceed the normal limit in 92.3, 26.3, and 23.0% of the patients with pancreatic cancer, benign biliary and pancreatic diseases, respectively, compared to healthy volunteers (Ozkan et al., 2006). In agreement with the previous study by Akashi et al., RCAS1 exhibited similar specificity to that of CA19-9 in pancreatic cancer, whereas RCAS1 presented a higher sensitivity (Ozkan et al., 2006). Both tumor markers presented similar predictive values of positive and negative tests for pancreatic cancer; however, RCAS1 levels were less influenced than CA19-9 ones by biliary stenoses (Ozkan et al., 2006). The median serum levels of RCAS1 also increased, as the tumor stage increased. Thus, RCAS1 proved a valuable serum marker for the diagnosis of pancreatic cancer, while both RCAS1 and CA19-9 were assumed to increase the diagnostic efficiency of each other in this type of cancer (Ozkan et al., 2006).

Furthermore, RCAS1 was compared with CEA and CA19-9 in 48 patients with pancreatic exocrine tumors (Yamaguchi et al., 2005). When the diagnosis of benign or malignant state was examined by one tumor marker, the sensitivity of RCAS1 alone was higher than that of CEA alone, and the specificity of RCAS1 alone was greater than that of CA19-9 alone (Yamaguchi et al., 2005). The combination of both RCAS1 and CA19-9 presented superior sensitivity than CA19-9 alone, RCAS1 alone, CEA alone, RCAS1 and CEA, as well as CA19-9 and CEA. These results further supported the previous data that the combination of RCAS1 and CA19-9 may be highly sensitive for pancreatic carcinoma (Yamaguchi et al., 2005). Indeed, Enjoji et al. suggested that RCAS1 may be a significant marker for biliary and pancreatic carcinomas, especially in cases where other markers, such as CA19-9 and CEA, are invalid (Enjoji et al., 2004). These authors also supported that if RCAS1 level increases in patients after curative treatments of cancers, a diligent search for a recurrence should be made (Enjoji et al., 2004). In this respect, a comprehensive review by the same research group thoroughly summarized the clinical application of RCAS1 antigen in hepatic and pancreaticobiliary diseases and offers useful suggestions for future studies

(Enjoji et al., 2005).

Lung neoplasia

Lung cancer patients with malignant pleural effusions exhibited significantly higher pleural fluid RCAS1 concentrations than non-malignant ones (Aoe et al., 2006). Moreover, patients with high pleural fluid RCAS1 levels were characterized by shorter mean survival than those with lower levels (Aoe et al., 2006). This relationship was remained significant in multivariate analysis, rendering pleural fluid RCAS1 levels as an independent prognostic factor in lung cancer patients with effusion. It was also shown that RCAS1 was not associated with other potential markers in pleural effusion, such as CEA, cytokeratin 19 fragment (CYFRA), sialyl SSEA-1 (SLX) and SCC antigen, which have limited reliability when considered separately (Aoe et al., 2006). These data deserve special attention, since differentiating malignant from non-malignant pleural effusions is a critical problem and conventional methods have proven inaccurate (Aoe et al., 2003). Moreover, cytologic examination of pleural fluid fails to detect tumor cells in 40-50% of malignant effusions, while blindly obtained pleural needle biopsy specimens offer little additional sensitivity (Fraser et al., 2003). In this regard, RCAS1 determination at the onset of pleural effusion may improve the diagnostic yield.

In another study, Hiraki et al. evaluated the diagnostic value of soluble RCAS1 protein in 38 patients with pleural malignant mesothelioma (Hiraki et al., 2005). RCAS1 concentrations in pleural fluid were significantly lower than those in lung cancer, supporting evidence that RCAS1 levels in pleural fluid may be a useful tool for the diagnosis of this disease (Hiraki et al., 2005).

Ovarian neoplasia

Sonoda et al. assessed by ELISA serum RCAS1 levels in samples from 75 healthy blood donors and 97 patients, 36 with ovarian benign tumors and 61 with ovarian cancer (Sonoda et al., 2007b). Ovarian cancer patients presented significantly higher serum RCAS1 concentrations than did healthy blood donors and patients with benign tumors. RCAS1 levels were significantly different according to the histopathological subtype for both benign and malignant tumor patients (Sonoda et al., 2007b). Carcinosarcomas, mucinous endometrioid and serous adenocarcinomas presented the highest RCAS1 levels. RCAS1 values were also significantly associated with the response to treatment. Moreover, the growth inhibition of K562 cells, which express the putative RCAS1 receptor, was assessed by tetrazolium salt (WST-1) assay using serum samples to clarify the biological function of RCAS1 (Sonoda et al., 2007b). WST-1 assay showed that serum obtained from ovarian carcinoma patients induced K562 cell growth inhibition, an effect that was partially recovered after

RCAS1 immunodepletion. Thus, these data supported substantial evidence that serum RCAS1 concentration may be a biomarker of ovarian cancer by virtue of its ability to predict results of treatment and inhibit immune cell growth (Sonoda et al., 2007b).

Endometrial neoplasia

Serum RCAS1 levels were significantly higher in endometrial cancer patients than in healthy blood donors (Sonoda et al., 2006). However, serum RCAS1 levels showed no significant associations with clinical stage, tumor histopathological type and grade and lymph node metastasis (Sonoda et al., 2006).

Cervical neoplasia

Uterine cervical cancer patients were shown to be characterized by significantly higher serum RCAS1 levels than healthy controls (Sonoda et al., 2006). In addition, patients with cervical adenocarcinoma presented significantly higher serum RCAS1 levels than those with SCC. RCAS1 levels were significantly reduced in patients presenting a positive response to treatment, while they increased in patients whose tumor sizes clearly increased (Sonoda et al., 2006). WST-1 assay showed that patients' serum induced K562 cell growth inhibition, an effect that was partially recovered by RCAS1 immunodepletion. Moreover, the number of peripheral blood lymphocytes exhibited an inverse relation to serum RCAS1 levels. In view of the above findings, it was assumed that serum RCAS1 levels may be considered as a critical biomarker of uterine cancer, as a result of its potential to predict results of uterine cancer treatment and inhibit the growth of immune cells (Sonoda et al., 2006).

Skin neoplasia

In a case report study performed on a 66-year-old male with primary skin lesion due to extramammary Paget's disease, serum RCAS1 level was found elevated before therapy and fell during and after therapy (Enjoji et al., 2003). Thus, assessment of serum RCAS1 levels was assumed to be valuable for estimating the viability of extramammary Paget's disease and may exhibit great potential clinical value as a sensitive systemic tumor marker compared to CEA (Enjoji et al., 2003).

Conclusions

It is certainly well-established that RCAS1 is overexpressed in various tumors and thus it may affect many aspects of cancer biology, such as differentiation, proliferation, invasion and angiogenesis. In this context, elevated tissue RCAS1 expression was reported in malignant tumor states in several organs, including uterus, skin, liver, gallbladder, stomach, lung, colon, breast, esophagus and pancreas. Despite the variety of

methods used and the criteria of tissue RCAS1 expression definition, most studies suggested that RCAS1 is implicated in several human malignancies. Tissue RCAS1 expression was associated with important clinicopathological parameters for patients' management. In oral and esophageal SCCs, colorectal, gallbladder lung and prostate carcinomas and gliomas, tissue RCAS1 expression was correlated with disease stage. In gastrointestinal carcinomas, such as gastric, GIT or GIMT and colorectal carcinomas, RCAS1 expression was correlated with lymph node metastasis.

Recent studies have also suggested that tumor cells may obtain the ability to evade immune surveillance by several mechanisms, including RCAS1 up-regulation, and that RCAS1 positive tumor cells may induce apoptosis to their surrounding TILs. In this regard, several studies provided evidence for a possible relationship between RCAS1 expression and the frequency of TILs apoptosis in human neoplasia. In fact, the proportion of apoptotic TILs was significantly higher in RCAS1-positive than in -negative tumors of the oral cavity, stomach, colon, lung, breast, cervix and brain. These findings supported substantial evidence that tumor-derived RCAS1 may induce apoptosis of TILs through the RCAS1-receptor, leading to immune cell depletion in several types of neoplasia.

Histological RCAS1 expression was also shown to correlate with the survival rate and prognosis in several human malignancies, such as esophageal SCC, gastric, GIMT, gallbladder, colorectal, liver, breast, lung and cervical carcinomas, as well as gliomas. In these types of neoplasia, multivariate analysis indicated that enhanced tissue RCAS1 expression was an independent negative predictor for patients' survival. The data seems more controversial in the case of oral SCCs, pancreatic and prostatic neoplasia, while no significant prognostic value was reported for HCCs and ovarian neoplasia. However, it should be noted that the available studies so far did not use a standard criterion for RCAS1 expression definition. Most studies evaluated RCAS1 immunohistochemical expression based on the percentage of RCAS1-positive malignant cells; however, they used different cutoff points for RCAS1 expression definition. On the other hand, a lower number of studies considered the intensity of staining to investigate the relationships of RCAS1 immunoreactivity with histopathological parameters and patients' survival, while others used both the extent and the intensity of RCAS1 staining incorporated in an immunohistochemical score, which further varied regarding the cutoff points for RCAS1 expression definition. Thus, it is extremely important to elucidate a standard criterion, as well as to establish the precise cutoff points for RCAS1 expression definition in order to be considered as a diagnostic and prognostic marker in routine clinical settings. Moreover, some of the studies were conducted on a limited number of cases, which further increases the probability of errors in the statistical analysis of the data, while the evidence obtained from studies performed on large clinical

samples are far more reliable.

Recent studies have also revealed that serum RCAS1 concentrations determined by ELISA could be a useful marker in screening procedure for several malignancies, including pancreatic, lung, ovarian, endometrial and cervical cancer. Soluble RCAS1 in pleural fluid was also shown to be a useful marker for the diagnosis of malignant mesothelioma. However, the clinical application of circulating RCAS1 concentrations in biofluids as a marker in the management and prognosis of tumor malignancies needs to be further explored, since the data so far are still extremely limited.

Taken together, the available data so far suggests that histological RCAS1 expression may have a significant value as a biomarker for the diagnosis and prognosis of human neoplasia. Further exploration of the biological functions of RCAS1, as well as evaluation of circulating RCAS1 levels in biofluids, could contribute to the development of new therapeutic strategies against human malignancies. In this context, basic research should be orientated to the delineation of RCAS1 functions and interactions with cancer related molecules, as well as to the determination of circulating RCAS1 levels during and after the treatment with the aim of recognizing patients' recurrence. In addition, as RCAS1 appears to play a crucial role in tumor immune escape, it may be considered as a promising target for future (gene) therapeutic approaches. In this aspect, the recent evidence that RCAS1 siRNA suppressed RCAS1 mRNA and protein expression and delayed tumor growth, in vivo, has unfolded new perspectives for the development of future inhibitors to target RCAS1 receptor.

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RCAS1 and human neoplasia

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