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RAS Mutations in Thyroid Cancer

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Key Words. RAS • Thyroid cancer • Thyroid nodules • Molecular markers

Learning Objectives

Explain the role of RAS mutations in thyroid carcinogenesis.

Describe the histologic spectrum and prognostic implications of RAS-associated thyroid neoplasms.

Explain the role of RAS mutation testing in patient care management algorithms.

ABSTRACT

In recent years, our understanding of the genetic alterations underlying thyroid oncogenesis has greatly expanded. The use of molecular markers, including *RAS*, in the management of thyroid carcinoma is also increasing. This review summarizes the current literature surrounding *RAS* and discusses its potential as a diagnostic and prognostic indicator in the management of thyroid cancer. ***The Oncologist*** 2013;18:926–932

Implications for Practice: In recent years, our understanding of the molecular mechanisms underlying thyroid oncogenesis has greatly expanded. Further, the use of some molecular markers in the clinical management of thyroid cancer is increasing. Mutations in *RAS* represent the second most common genetic event in thyroid neoplasia. However, the significance of *RAS*-positive mutation status and the biological behavior of thyroid carcinomas that harbor *RAS* are not completely understood. The purposes of this review are to clarify the current literature surrounding *RAS* mutations in thyroid cancer and to examine the potential utility of *RAS* as a diagnostic tool to predict the presence of malignancy, thus altering subsequent clinical management. In addition, the prognostic value of *RAS* positivity in predicting the risk for tumor aggressiveness, recurrence, and mortality is discussed.

INTRODUCTION

Thyroid cancer (TC) is the most common endocrine malignancy, and its incidence is on the rise [1]. Tumors of follicular epithelial cell origin account for the vast majority of these cancers, and of these, well-differentiated papillary thyroid cancer (PTC) and follicular thyroid cancer (FTC) account for 95%; whereas, poorly differentiated thyroid cancer (PDTC) and anaplastic thyroid cancer (ATC) are observed far less frequently [2]. In recent years, our understanding of the molecular mechanisms underlying thyroid oncogenesis has greatly expanded, thus enabling differentiation of thyroid tumors based on characteristic genetic alterations in addition to traditional histologic criteria [3]. The most clinically relevant markers to date include point mutations in *BRAF* and *RAS* and *RET/PTC* and *PAX8/PPARγ* rearrangements. PTC is known to harbor *BRAF* most commonly, followed by *RAS* and *RET/PTC*, whereas FTC is characterized by the presence of either *RAS* or *PAX8/PPARγ* [3–6].

The use of these molecular markers in the management of thyroid carcinoma is increasing. *BRAF* is perhaps the most studied of the markers and has emerged as an important diagnostic and prognostic tool. For example, the finding of *BRAF* in a thyroid nodule with indeterminate cytology is associated with a PTC risk of nearly 100%, and further, patients with *BRAF*-positive PTC are more likely to have aggressive and recurrent disease [7–11]. Likewise, *RAS* represents the second most common genetic mutation in TC and was first implicated in thyroid neoplasia more than two decades ago [12]. Despite this, the significance of *RAS*-positive mutation status and the biological behavior of thyroid carcinomas that harbor *RAS* are still not completely understood. In part, this uncertainty is because *RAS* mutations have been reported in the full spectrum of thyroid neoplasms ranging from benign follicular adenomas to anaplastic carcinomas, thus obscuring its true clinical relevance [4, 5].

Therefore, the purpose of this review is to clarify the current literature surrounding *RAS* mutations in TC. Specifically, we will discuss the prevalence and isoform pattern of *RAS*, the pathogenesis of *RAS*-mediated oncogenesis, and the potential utility of *RAS* as a diagnostic and prognostic tool in the management of TC.

Prevalence and Isoform Pattern of *RAS* Mutations

The *RAS* gene encodes a family of three highly homologous isoforms: *NRAS, HRAS,* and *KRAS*. These 21-kDa membrane-

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Table 1. Reported overall frequency of *RAS* mutations in thyroid neoplasms

Reference	FA	FTC	HA	HC	PTC	PDTC	ATC
Lemoine et al. (1989) [18]	33% (8/24)	40% (4/10)	–	–	–	–	60% (6/10)
Namba et al. (1990) [24]	25% (6/24)	0% (0/3)	–	–	21% (3/14)	–	–
Esapa et al. (1999) [21]	18% (7/38)	44% (4/9)	0% (0/3)	–	8% (1/13)	–	100% (1/1)
Basolo et al. (2000) [25]	–	40% (2/5)	–	–	10% (3/31)	18% (8/44)	60% (3/5)
Garcia-Rostan et al. (2003) [27]	–	11% (2/19)	–	–	7% (2/30)	55% (16/29)	52% (15/29)
Nikiforova et al. (2003) [6]	48% (11/23)	52% (16/33)	8% (1/13)	11% (2/19)	–	–	–
Vasko et al. (2003) [19]	20% (9/46)	21% (7/34)	–	–	–	–	–
Zhu et al. (2003) [26]	–	–	–	–	17% (13/76)	–	–
Liu et al. (2004) [20]	8% (1/12)	38% (3/8)	0% (0/5)	–	0% (0/42)	–	0% (0/2)
Fukahori et al. (2012) [28]	30% (12/40)	57% (33/58)	–	–	–	–	–
Summary total	26% (54/207)	40% (71/179)	5% (1/21)	11% (2/19)	11% (22/206)	33% (24/73)	53% (25/47)

Abbreviations: –, not evaluated; ATC, anaplastic carcinoma; FA, follicular adenoma; FTC, follicular carcinoma; HA, Hurthle cell adenoma; HC, Hurthle cell carcinoma; PTC, papillary carcinoma; PDTC, poorly differentiated carcinoma.

associated proteins play a central role in the transduction of signals from tyrosine kinase and G protein-coupled receptors to effectors of the MAPK and PI3K-AKT signaling pathways, which mediate cell differentiation, proliferation, and survival [13, 14]. Under normal conditions, *RAS* activity is tightly regulated by GTP-mediated hydrolysis of activated GTP-bound *RAS* to inactivated GDP-bound *RAS*. Point mutations produce oncogenic alleles of *RAS* that exhibit either increased affinity for GTP (codons 12 and 13) or inhibition of autocatalytic GTP-ase function (codon 61). Both mechanisms result in constitutive, aberrant activation of the downstream MAPK and PI3/AKT signaling pathways, a critical event in thyroid tumorigenesis [4, 9, 12, 15, 16].

Thyroid neoplasms are unique in that they have been associated with all three mutant isoforms of the *RAS* gene, although most series demonstrate predominance of *NRAS61* [6, 17–23]. Further, the literature cites overall frequencies of *RAS* mutations in up to 48% of benign follicular adenomas (FA), 57% of FTC, and 21% of PTC (Table 1) [6, 18–21, 24–28]. However, the overall prevalence and pattern of specific isoform frequency varies significantly among reports. In part, this is because existing data are comprised mostly of small studies that often differ with respect to methodological criteria. For example, *RAS* prevalence is generally lower when analysis is limited to studies that use direct sequencing—the gold standard—either exclusively or for confirmation of mutation identification. This was illustrated by Vasko et al., who noted a significantly higher overall rate of mutation detection (17% vs. 12%, $p < .01$), particularly with respect to overestimation of *HRAS61*, when direct sequencing was not used [19].

To address these methodological limitations, Liu et al. performed a meta-analysis of 86 *RAS* tumors described in a restricted group of publications. In all the selected studies, tumors underwent direct sequencing for mutation identification and were routinely screened for all three mutant isoforms (*H-, K-,* and *NRAS*) in the same tumor. The study reported that mutations involving *NRAS* at codon 61 were by far the most numerous, accounting for 67% of all *RAS* mutations in the series [20]. This finding was corroborated by another pooled analysis of 22 studies with similar inclusion criteria, in which *NRAS61* accounted for 88% of *RAS* mutations [19]. Both pooled analyses further concluded that *RAS* mutations were more prevalent in FTC than in benign FA, and were relatively uncommon in PTC. Liu et al. reported frequencies of 27%, 15%, and 6% for FTC, FA, and PTC, respectively, and Vasko et al. reported a frequency of 25%, 14%, and 5% for FTC, FA, and PTC, respectively [19, 20].

The almost exclusive occurrence of *RAS* mutations in follicular tumors and their rare appearance in PTC has also been suggested in other studies; however, there is increasing evidence that *RAS*-positive PTC may be restricted to the follicular variant subtype (FVPTC), which possesses the follicular growth pattern and architecture typical of follicular tumors [21, 26, 29]. For example, a recent study conducted to examine the prevalence of *RAS* mutations in 30 cases of FVPTC and 46 cases of non-FVPTC demonstrated that none of the non-FVPTC were *RAS*-mutation positive, but 43% of FVPTC were *RAS*-mutation positive [26]. This suggests that the occurrence of *RAS* mutations in PTC may be underestimated in study samples that do not include the follicular variant subtype.

Reported prevalence may also be influenced by the inclusion of, or failure to distinguish, oncocytic variants of follicular tumors. Hurthle cell carcinoma (HC) and its benign counterpart, Hurthle cell adenoma (HA), account for one third of all follicular lesions and are far less likely to harbor *RAS* mutations than conventional FTC and FA [6, 20, 30]. A recent study examining a series of 33 FTC, 23 FA, 19 HC, and 13 HA detected *RAS* mutations in 52% of FTC and 48% of FA, but in only 11% of HC and 8% of HA [20]. Furthermore, the isoform pattern between conventional FTC and HC also differed with mutations of *NRAS* at codon 61 common in the former but absent in the latter. Hurthle cell tumors exhibited mutations only in *HRAS* at codon 61 and *KRAS* at codon 12, once again underscoring the importance of the subtype histologic variant when interpreting overall and individual isoform prevalence data [20].

Pathogenesis of *RAS*-Positive Thyroid Carcinoma

Although the ability of activated mutant *RAS* to induce thyroid neoplasia in both in vivo and in vitro experimental studies has been known for a number of years [31, 32], the clinical appli-

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cability of detecting this mutation has only recently been described. The current understanding is that *RAS* mutations that occur in well differentiated thyroid cancer (WDTC) represent distinct molecular events that are mutually exclusive with other genetic alterations that occur in FTC and PTC. It is postulated that up to 85% of conventional FTC develop along one of two described molecular pathways involving either *RAS* mutation or *PAX8-PPARγ* gene rearrangement [6]. A recent investigation reported that 49% and 36% of conventional FTC were positive for *RAS* and *PAX8-PPARγ*, respectively [6]. Furthermore, only 1 out of 33 FTC in this series had both alterations, supporting the concept of two discrete, essentially non-overlapping molecular mechanisms [6]. With respect to papillary cancer, *RAS* mutations are generally restricted to the follicular variant subtype, as previously mentioned [21, 23, 26]. Furthermore, the most common alterations found in non-FVPTC, *BRAF* V600E and *RET/PTC*, are infrequent in FVPTC, again suggesting a distinct *RAS*-mediated mechanism for tumorigenesis in PTC [26, 33].

The precursor lesion for *RAS*-mediated development of FTC and FVPTC is currently hypothesized to be *RAS*-positive FA [3, 4, 6]. This theory stems from the consistent demonstration of *RAS* mutations in FA, a feature that is not typical of other well-described genetic events in TC, such as *PAX8-PPARγ, BRAF* V600E, and *RET/PTC* mutations [6, 18]. A recent series of *RAS*-positive tumors demonstrated distribution along the full histologic spectrum from benign FA, encapsulated and indolent FVPTC, encapsulated FVPTC with capsular invasion, non-encapsulated infiltrative FVPTC, FTC, and ATC [23]. Similarly, in a group of follicular tumors, *RAS* mutations were found in both benign FA, with no morphologic indicators of malignancy, and in FTC that ranged from minimally, overtly, and widely invasive [6].

The above-mentioned studies seem to suggest that *RAS* mutation occurs as an early event in FA and may increase the potential for malignant transformation. Further, there are also data to suggest that *RAS* may predispose WDTC to subsequent de-differentiation into poorly differentiated thyroid cancer (PDTC) and ATC [34]. *RAS* is present at all stages of tumor differentiation, which is not a characteristic feature of other molecular alterations [18, 25]. For example, in one of the first studies to examine the prevalence of *RAS* in a full spectrum of thyroid tumors, *RAS* mutations were found in 33% of FA, 53% of WDTC, and 60% of ATC [18]. In addition, PDTC and ATC seem to maintain *RAS* activation while also gaining mutations in *p53*, *beta-catenin*, *PTEN*, and/or *PI3KCA* genes [35–38]. The acquisition of these "late" genetic events in *RAS*-positive tumors further supports the notion of stepwise *RAS*-mediated oncogenesis, in which an early transformative mutation in *RAS* predisposes to future molecular alterations that promote development of cancer and subsequent de-differentiation. The natural history and progression of *RAS*-positive neoplasms is unfortunately difficult to confirm definitively, and these theories remain speculative.

Diagnostic Utility of *RAS* Mutation

Thyroid nodules are common, and the clinical challenge is to identify the 5%–15% that harbor or that are at increased risk for developing malignancy [39]. Fine-needle aspiration biopsy (FNAB) is the recommended diagnostic procedure of choice but can yield an indeterminate cytology result up to 30% of the time [40, 41]. Patients with indeterminate cytology may ultimately require diagnostic surgery to exclude malignancy; however, this carries with it the potential for operative complications and undue health care costs [42]. As a result, much recent attention has focused on improving the diagnostic accuracy of FNAB through the use of adjunctive molecular testing for those with indeterminate cytology, a practice that is supported by current American Thyroid Association guidelines [39, 43].

Molecular testing should occur as part of a panel which is an effective strategy because the most commonly described alterations, *BRAF, RAS, RET/PTC*, and *PAX8-PPARγ*, are not only mutually exclusive but can be found in up to 70%–80% of TC [4, 5]. Three recent studies that examined the utility of concurrent testing of FNAB specimens for the above-mentioned alterations found that the presence of any mutation was a strong predictor of cancer, with histologic confirmation of malignancy in 89%–97% of specimens [7, 8, 44]. Yet, unlike *BRAF* and *RET/PTC*, which nearly always confer a malignant diagnosis, the predictive value of a detected *RAS* mutation is less definitive [7, 8, 44, 45]. This is because *RAS* mutations are consistently found in benign FA, leading some to even suggest that *RAS*-positive FA should be classified as "false-positive" molecular results [44]. However, this contrasts with the prevailing notion that *RAS*-positive FA is likely a precursor to *RAS*-positive follicular-patterned cancer [6, 22, 23, 46].

In tumors of follicular cell origin, *RAS* mutations are essentially restricted to FA, FTC, and FVPTC which are difficult to differentiate as benign or malignant based on cytology alone, and are therefore often indeterminate by FNAB. It is precisely in this group of FNAB results that *RAS* mutation testing may be most clinically useful. In fact, in a recent series of 67 prospectively identified *RAS*-positive thyroid nodules, cytology was malignant in 3%, benign in 3%, but indeterminate in 94% [23]. Similarly, Nikiforov et al. evaluated a large series of FNAB specimens with indeterminate cytology and found that *RAS* was the most common mutation detected (72%), followed by *BRAF* (21%), *PAX8-PPARγ* (6%), and *RET/PTC* (1%) [7]. Further, in this study, the probability of cancer associated with *RAS*-positive mutation status was 85%, which is consistent with other reports in the literature that range from 74% to 88% [7, 8, 43, 45].

> The markedly elevated increased risk for cancer (~85%) when *RAS* mutation is present potentially alters initial surgical management for the group of patients with otherwise indeterminate cytology.

Although identification of *RAS* mutation in the FNAB specimen is not 100% predictive of cancer, it is certainly highly suggestive of either FTC or FVPTC and thus has significant diagnostic value. The markedly elevated increased risk for cancer (~85%) when *RAS* mutation is present potentially alters initial surgical management for the group of patients with otherwise indeterminate cytology [7]. Even if an *RAS*-positive indeterminate nodule is histologically con-

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Table 2. *RAS* mutation and poor outcome

Reference	N	Tumor	*RAS* positive	Distant metastases	Recurrence	Death	Comments
Hara et al. (1994) [52]	91	PTC	13/91 (14%)	Y	Y	Y	Prevalence of *RAS* positive higher in patients with distant metastasis (28% vs. 8%). Kaplan-Meier analysis: Higher recurrence rate ($p <$ 0.01) and mortality ($p < .05$) in *RAS* positive vs. *RAS* negative
Karga et al. (1991) [56]	14	FTC	2/14 (14%)	Y	–	–	2/2 patients with *RAS*-positive FTC had bone metastasis
Basolo et al. (2000) [25]	5	FTC	2/5 (40%)	Y	–	–	2/2 patients with *RAS*-positive FTC had bone metastasis
Garcia-Rostan et al. (2003) [27]	107	30 PTC, 19 FTC, 29 PDTC, 29 ATC	35/107 (33%)	Y	–	Y	Mortality was higher (74% vs. 32%) and distant metastasis was more likely (49% vs. 24%) in patients with *RAS*-positive vs. *RAS*-negative cancer
Volante et al. (2009) [53]	65	PDTC	15/65 (23)	–	–	Y	Kaplan-Meier analysis: Higher mortality ($p = .004$) in patients with *RAS*-positive vs. *RAS*-negative tumors
Fukahori et al. (2012) [28]	58	FTC	33/58 (57)	Y	N	Y	Significant association (χ^2) between *NRAS61*-positive status and distant metastasis ($p = .02$) and any *RAS*-positive status and death ($p =$ .042); χ^2 not significant for recurrence

Abbreviations: –, not evaluated; ATC, anaplastic carcinoma; FTC, follicular carcinoma; PDTC, poorly differentiated carcinoma; PTC, papillary carcinoma.

firmed as FA, some would argue that FA has an increased risk for malignant degeneration and may be best treated by surgical resection.

Although it is not the focus of this discussion, it should also be mentioned that *RAS* mutation has been implicated in the pathogenesis of non-familial, *RET*-negative medullary thyroid cancer (MTC), with a reported prevalence ranging from 17% to 81% [47–49]. In the context of sporadic MTC, *RAS* and *RET* are thought to represent alternative genetic events, and thus knowledge of *RAS* mutation status has potential diagnostic value and can aid in the selection of targeted therapies [50].

Prognostic Utility of *RAS*

Most patients with WDTC have an excellent prognosis, with an average 20-year survival following tumor resection above 90% [51]. The clinical challenge remains in identifying the subset of tumors that have aggressive biological behavior and therefore have a negative impact on patient morbidity and mortality. To date, few publications have addressed the prognostic significance of *RAS*-positive TC, and no universal conclusions can be drawn at this time. There is, however, some evidence to suggest that a select group of patients with *RAS*-positive WDTC may be at risk for *RAS*-mediated tumor de-differentiation, distant metastases, and shortened survival [25, 27, 28, 52, 53].

Poorly differentiated and anaplastic subtypes of TC typically arise from stepwise de-differentiation of PTC and FTC, a process that may be facilitated by mutation in the *RAS* oncogene [2, 3]. Evidence in favor of this theory stems in part from the in vitro finding that mutant *RAS* can promote chromosomal instability [54]. Further, it has been noted that well differentiated FTC and PTC with focal areas of poorly differentiated histology are often *RAS* mutation positive. Zhu et al. reported that all identified cases of PTC with focal areas of poorly differentiated carcinoma were of the follicular variant subtype, and that 67% of these cases harbored mutations in *RAS* [26]. Similarly, Nikiforova et al. found that FTC with focal

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Given the high probability of cancer, an *RAS*-positive lesion with indeterminate cytology could be considered for initial upfront total thyroidectomy to eliminate the need for, risks of, and costs of re-operative completion thyroidectomy.

areas of poorly differentiated histology contained mutated *RAS* [6].

In addition to the correlation between *RAS* and loss of histologic features characteristic of WDTC, a high frequency of *RAS* mutations in PDTC and ATC has been reported in many observational studies (Table 1) [18, 21, 25, 27]. An early study by Lemoine et al. found *RAS* mutations in 60% of undifferentiated ATC, and a more recent investigation described a similar prevalence of *RAS* in 55% of PDTC and 52% of ATC [18, 27]. Another study by Volante et al. sought to characterize the individual frequency of molecular alterations in a group of 65 PDTC diagnosed using strict Turin criteria and reported a predominance of *RAS* mutations in 23% of cases ($n = 15$) [53]. In contrast, only one mutation was identified in *BRAF* V600E and no mutations were detected in *KRAS*, *RET/PTC*, or *PAX8-PPARγ*, again suggesting the exclusive potential ability of *RAS* to predispose to de-differentiation and/or anaplastic transformation [53].

It has also been suggested that *RAS* may confer a more aggressive phenotype in some cases, increasing a patient's risk for tumor recurrence, distant metastases, and death (Table 2) [25, 27, 28, 52, 53, 55, 56]. In 91 cases of PTC, Hara et al. found *NRAS61* mutation in 14% ($n = 13$) of patients overall, with a significantly higher incidence of *RAS* in patients with distant metastases than in those without (28% vs. 8%, $p = .01$), and in patients who died versus those who were still alive (33% vs. 10%, $p = .02$) [52]. Kaplan-Meier survival analysis further revealed that patients in the *RAS*-positive group experienced significantly greater mortality and recurrence than those in the *RAS*-negative group [52].

Several additional studies drew similar conclusions regarding *RAS* as a marker for poor prognosis, although the sample size in each study was small and mutation testing was performed only on selected patients. Manenti et al. and Karga et al. reported an association between *RAS* mutations and hematogenous bone metastases [55, 56]. Garcia-Rostan et al. examined a heterogeneous group of TC, including WDTC, PDTC, and ATC, and found that *RAS* was an independent predictor of poor survival even when restricting the analysis to differentiated cancers [27]. Finally, Fukahori et al. reported that *RAS* was significantly associated with both distant metastases and death in their series of patients with FTC [28].

Potential Management Algorithm Incorporating *RAS* Mutation Status

Molecular testing for *RAS* mutation of the FNAB specimen can provide useful clinical information that can be of diagnostic value and may alter patient management. As mentioned previously, several studies have demonstrated that the finding of *RAS* mutation in a thyroid nodule is highly suggestive of malignancy, with predictive values ranging between 74% and 88% [7, 8, 43, 44]. Because *RAS* mutations are found with high frequency in nodules with indeterminate cytology, this added information has a significant impact, particularly when diagnostic lobectomy is the next step in management. Given the high probability of cancer, an *RAS*-positive lesion with indeterminate cytology could be considered for initial upfront total thyroidectomy to eliminate the need for, risks of, and costs of re-operative completion thyroidectomy. There is also evidence that *RAS*-positive cancers are frequently bilateral, further supporting the choice of upfront total thyroidectomy for these patients [23]. A study by Gupta et al. in 46 patients with *RAS*-positive malignancy found bilateral cancer in 43% ($n = 20$): 45% of these contralateral cancers were positive for *RAS* mutation, 5% were positive for *BRAFV600E*, and 50% did not undergo molecular testing. Notably, the majority of patients with bilateral cancer underwent diagnostic lobectomy as their initial procedure, resulting in the need for a second operative procedure in most [23].

In some circumstances when the diagnosis of cancer is known preoperatively, total thyroidectomy is indicated. In the case of PTC, central neck dissection may also be considered because of the propensity of PTC to spread to central compartment lymph nodes and its association with recurrent disease [39, 57]. However, *RAS*-positive PTC is nearly always FVPTC, which has been characterized by a lack of central compartment lymph node metastases in several studies [23, 26, 33, 58]. Thus, central neck dissection in *RAS*-positive cancers can likely be deferred in the absence of clinically evident or suspicious nodal disease.

Conclusion

Mutations in *RAS* represent the second most commonly identified genetic alteration in TC. *RAS* mutations are primarily found in follicular-patterned tumors, including FA, follicular cancer, and the follicular variant of papillary cancer. There is increasing evidence that *RAS* mutation status has significant diagnostic utility when used concurrently with FNAB. This is particularly true for lesions with indeterminate cytology, for which the detection of *RAS* may have a potential impact on initial surgical management. Although some data suggest that *RAS* mutation positivity is associated with tumor progression to histologic subtypes associated with poor prognosis, future studies will be needed to determine further whether prospective *RAS* testing has equal prognostic significance.

Author Contributions

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