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Expression of *ras* proto-oncogenes: regulation and implications in the development of human tumors

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Aberrant expression levels of the *ras* genes is a common event in human tumors. Transcriptional regulation of the H-*ras* proto-oncogene, the most well studied member of the *ras* family, occurs through nuclear factors that recognize elements in the promoter region of the gene, in the first and fourth intron and in the variable tandem repeat (VTR) region unit and involves alternative splicing and specific methylation patterns, as well. Overexpression of the Ras p21 protein is detected in a variety of human tumors, including neuroblastomas, esophageal, head and neck, laryngeal, thyroid, lung, liver, intestinal, gastric, colorectal, breast, bladder, endometrial, ovarian tumors and leukemias. Levels of the Ras p21 protein are often of great prognostic and clinical significance and controling the expression of *ras* genes provides a useful target for gene therapy treatments.

1. Introduction

The *ras* family of cellular oncogenes, is one of the most frequently detected families of transformation-inducing genes in human solid tumors [1–3]. Three functional *ras* genes have been identified and characterized in the mammalian genome, H-*ras*1, K-*ras*2 and N-*ras*, as well as two pseudogenes, H-*ras*2 and K-*ras*1. All three *ras* genes have a common structure with a 5′ non-coding exon (exon-I) and four coding exons (exons I–IV). The introns of the genes differ widely in size and sequence, with the coding sequences of human K-*ras* spanning more than 35 kb, while those of N-*ras* and H-*ras* span approximately 7 and 3 kb, respectively. The K-*ras* gene has two alternative IV coding exons, thus encoding two proteins, K-RasA and K-RasB [4], the K-RasB form being more abundant. The H-*ras* gene also has an alternative fourth exon [5]. The exon is 82 bp long in the human and rodent gene and encodes for 20 amino-acids. In addition, H-*ras* has a variable tandem repeat sequence (VTR), located downstream of the polyadenylation signal, which exhibits an enhancer activity [6,7]. The H-*ras*, N-*ras* and K-*ras*A proteins are 189 amino acids long, whereas K-*ras*B is shorter by one amino acid. They all have a molecular weight of 21 kDa and are termed p21 proteins.

Comparison among the p21 proteins encoded by all three human *ras* genes, leads to similar conclusions: they are identical at the 86 N-terminal amino acid residues, they possess an 85% homology in the next 80 amino acid residues and diverge highly at the rest of the protein molecule, with the exception of the four C-terminal amino acids which share the common motif CAAX-COOH (C, cysteine 186; A, aliphatic amino acid-leukine, isoleukine or valine; X, methionine or serine) [2,3]. The Ras protein is synthesized in the cytoplasm on free ribosomes as pro-p21, undergoes a series of post-translational modifications at the C-terminus increasing the hydrophobicity of the protein and associates with the inner face of the plasma membrane [3]. Sequences at the C-terminus are essential for membrane association and the conserved Cys 186 is required to initiate the post-translational modifications of pro-p21 [8].

Ras is a GTP/GDP binding protein, it has intrinsic GTPase activity and is involved in multiple signal transduction pathways [9]. The Ras p21 protein interacts directly with the Raf oncoprotein to recruit the MAP kinases and their subordinates. In this way, a mitogenic signal initiated by membrane receptors with tyrosine kinase activity is converted by p21 into a cascade of serine/threonine kinases that, depending on its intensity, duration and the intracellular conditions, results in cell differentiation or cell division [10,11].

The *ras* gene family is frequently implicated in human tumors by four different mechanisms: mutation of *ras* proto-oncogenes [12,13], gene amplification [14], insertion of retroviral sequences [15] and alterations in regulation of transcription. With the exception of mutations, all other mechanisms result in activating the transforming properties of *ras* genes by quantitative mechanisms. Overexpression of the mutant T24 H-*ras* oncogene may cause oncogenic transformation of early passage rodent cells [16] and, in the presence of strong enhancer sequences, elevated expression of even the normal proto-oncogene can rescue the cells from senescence [17]. In addition, H-*ras* overexpression correlates with metastatic potential of cells in tissue culture [18] and increased levels of p21 have been detected in a variety of human cancers [19].

2. Transcriptional regulation of the human c-H-*ras*1 gene

Various reports have been published concerning transcriptional regulation of the H-, K-, and N-*ras* genes [20]. This review summarizes our knowledge on regulation of expression of the human H-*ras*1 proto-oncogene, which is the most well studied member of the *ras* family, and reports on the implication of aberrant expression of *ras* genes in the development of human tumors.

2.1. Regulation of the H-ras gene expression from promoter-like sequences

The three *ras* genes carry promoters with high GC content, that lack a characteristic TATA box and initiate RNA transcription at multiple sites [21]. These features are characteristic of housekeeping genes. The 6.6 kb *Bam*HI fragment encompassing the human c-H-*ras*1 gene and the regulatory elements has been analyzed in detail. Most promoter region studies have focused on the region upstream of the 5′ splice site of the first intron of the gene (nucleotides 1–577), although others consider the *Sst*I fragment (nucleotides 1–1054) that encompasses a part of the first intron as well, to be the gene promoter

[22]. A great number of regulatory elements in H-*ras* promoter was reported, but the results of different investigators were often controversial. Therefore, the exact position of the upregulator remains unclear and the confusion may be partly due to the use of non-equivalent analytical assays or constructs. According to Ishii et al. [23], the H-*ras* promoter contains six GC box sequences within the promoter which bind the Sp1 transcription factor (at nucleotides 262, 289, 340, 400, 509, 520), with the three sites closest to the RNA start sites being sufficient for full transformation activity (positions 289, 340, 400). Honkawa et al. [24], using deletion analysis of the H-*ras* promoter region by focus formation assay in NIH 3T3 cells, reported a minimum promoter region of 51 bp length (nucleotides 252–303), which was GC rich (78%) and contained a GC box (position 289). Lowndes et al. [25], using transient expression assays in which a series of mutants spanning the promoter region of H-*ras* were ligated to a promoterless chloramphenicol acetyl transferase (CAT) vector, located a 47 bp element, distinct of the one reported by Honkawa et al. (nucleotides 333–380), that upregulated the transcriptional activity of the promoter region by 20- to 40-fold and contained a GC box known to bind Sp-1 (position 340) and a CCAAT box (position 362), binding the NF-1 (CTF) factor. Jones et al. [26], also identified two NF-I binding sites, one strong at position 362 that was also noted by Honkawa et al, and one weak at position 327. Trimble and Hozumi [27], using CAT transfection experiments in CV-1 cells, also identified a 100 nucleotides region, about 1 kb upstream of the *ras* coding sequence (nucleotides 275–375), encompassing the consensus CCAAT box and two Sp-1 sites. On the contrary, Nagase et al. [28], using deletion mutants in CAT assays in CV-1 and A-431 cells, suggested that the presence of Sp-1 binding sites at specific positions may not be essential for promoter activity, but a number of Sp-1 binding sites in the region could be required. They also showed that the CCAAT-box (position 362) as well as a CACCC-box element at position 467, contributed in concert to the activity of the promoter region. They therefore suggested that any two of the GC boxes upstream of the CCAAT box (position 157, 262, 289, 340), also mentioned by Ishii et al. [21] and either one of the two Sp-1 binding sites downstream the CACCC box (position 509, 520), were sufficient for the maximal activity of the H-*ras* promoter. Lee and Keller [29], using recombinant plasmids encompassing internal deletions and point mutations of the promoter region in CAT assays, after transfection in HeLa cells, reported the 289 GC box, an unidentified element between nucleotides 195 and 248 and a new element CCGGAA directly upstream the GC box, as the most important regulatory elements. The CCAAT element at position 362 makes minor contributions and the remaining Sp-1 boxes little or no contribution to the transcription. Spandidos et al. [22], using recombinant plasmids in CAT activity experiments showed that AP-1-like proteins may play a role in control of H-*ras* transcription and identified four TPA responsive-AP-1 binding elements in the H-*ras* promoter, at positions 259, 309, 320, 424.

A great variety of the transcription initiation sites was also identified [20,25,28,29] using S1 nuclease analysis. The reported regulatory elements and factors that participate on the promoter activity of H-*ras*, as well as the major RNA start sites, are shown in Fig. 1.

2.2. Regulation of the H-ras gene expression from intronic sequences

Intronic sequences also play an important role in H-*ras* regulation. There is evidence that the mutant T24 *ras* 0.8 kb *Sst*I DNA fragment, when compared with the corresponding normal H-*ras* fragment, is a more potent activator of gene expression [30]. A structural basis for this difference was shown to be a 6 bp element in the mutant H-*ras* fragment, at position 680 in the first intron of the gene, proved to contain an Sp-1 binding site absent in the normal H-*ras* [31].

The human H-*ras* gene contains within its first intron sequences that partially match the p53 consensus binding site (at position 1198–1236 of the nucleotide sequence). It was determined that wild-type as well as mutant forms of the P53 protein specifically recognize the p53 element and that it shows enhancer activity when binding the wild-type P53, thus, suggesting that P53 regulates cell growth by coordinate transcription of genes that repress and promote cellular proliferation [32,33].

Zachos et al. [34], identified sequences in the 3′ end of the first intron (position 1261) and in the fourth intron of H-*ras* gene (position 3007), with high similarity with the glucocorticoid response elements (GRE) and estrogen response elements (ERE) consensus oligonucleotides, respectively. They also showed specific binding of the glucocorticoid and estrogen receptors at the respective H-*ras* elements, thus suggesting hormone regulation of H-*ras* proto-oncogene.

The first intron of the gene contains well conserved regions between human and rodents [35] and encompasses positive and negative elements influencing H-*ras* expression, respectively positively and negatively, possibly at post-transcriptional level [36]. It is noteworthy that both the GRE and the p53 element previously mentioned [32–34] are included at the conserved region of the intron (nucleotides 1170–1400), thus providing evidence for an essential role in regulation of the H-*ras* gene expression. In addition, the H-*ras* GRE is located in the first positive element (position 1261–1358).

The interactions of H-*ras* intronic DNA sequences with regulatory factors and the exact position of the positive and negative regulatory elements are shown in Fig. 2.

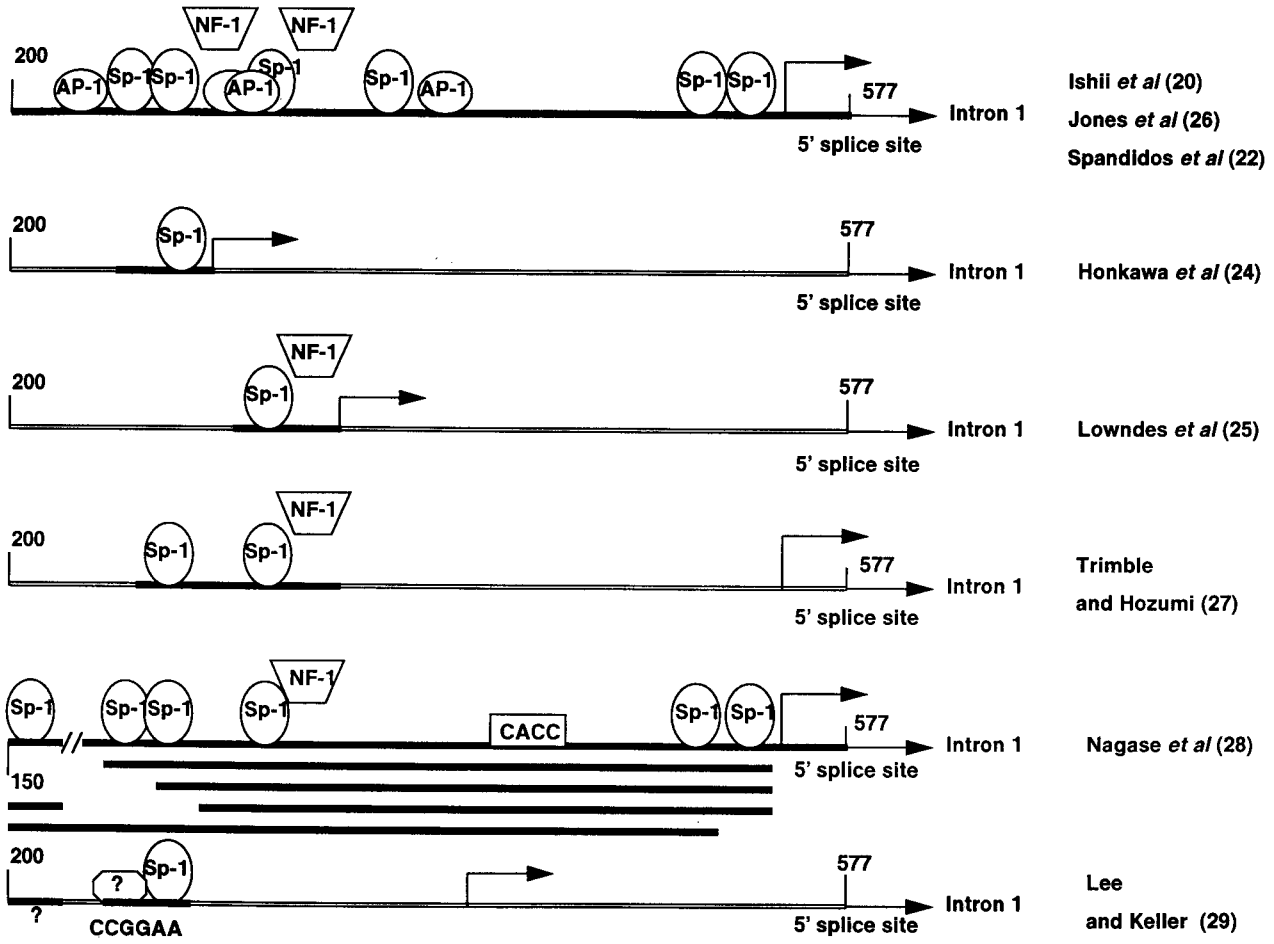

Fig. 1. Regulatory elements and transcription factors in the promoter of the human c-H-*ras*1 gene. A synopsis of the reported regulatory sequences and nuclear factors, essential for H-*ras* promoter activity. Solid bars, the reported 'core' sequences with promoter activity. Arrows, the major transcriptional initiation site. ?, unknown regulatory elements and factors. The sequence of the gene is given from position 200 to 577, the 5′ end of the first intron. Nucleotide numbering starts from the *Bam*HI cleavage site.

2.3. The role of the VTR in expression of the H-ras gene

Efficient expression of the H-*ras* gene requires sequences 3′ of the polyadenylation site, which include the VTR region. VTR sequences are shown to possess an endogenous enhancer activity of both normal and T24 mutant H-*ras* gene and of heterologous promoters as well, in both orientations [6,7]. The 28 bp repeat units of the minisatellite bind four proteins (p45, p50, p72 and p85) which are members of the *rell* NF-κB family of transcriptional regulatory factors [37], thus regulating transcription of the H-*ras* and nearby genes (Fig. 2).

2.4. DNA methylation affecting H-ras gene expression

CpG sites in vertebrates are either clustered in 0.5–2 kb regions called CpG islands, or are dispersed, in which case they are mostly methylated and constitute mutational hotspots [38]. The CpG islands are associated with gene promoters (e.g. H-*ras*) or coding regions (e.g. p16) and are unmethylated in autosomal genes. 5′ Methyl-cytosine can affect transcription by altering transcription factor binding to DNA either directly, for example binding of *trans*-acting proteins at AP-2 sites is inhibited [39], or indirectly, by enhanced binding of methylated DNA binding protein (MDBP) which stereochemically inhibits DNA binding of transcription factors [40]. The promoter region of the H-*ras* gene is undermethylated [41]. Furthermore, methylation of cis-elements decreases H-*ras* promoter activity in vitro [42] and inhibits the transforming activity of the oncogene [43].

2.5. Expression of the H-ras proto-oncogene is controlled by alternative splicing

A proportion of H-*ras* pre-mRNA is spliced to incorporate an alternative exon, termed IDX (intron D exon), which contains an in-frame translational termination codon that prevents expression of the genetic

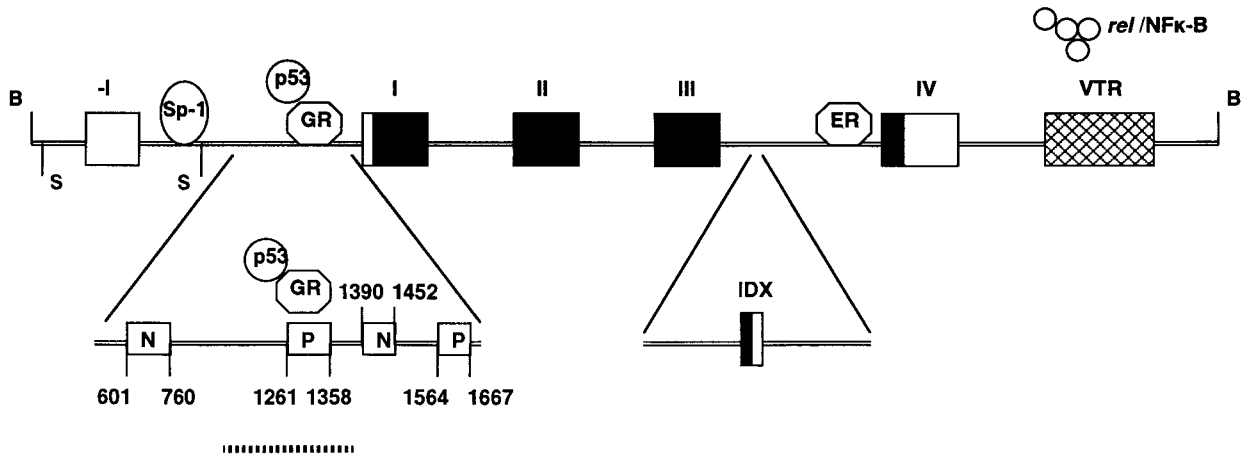

Fig. 2. Regulatory elements and transcription factors in the intronic sequences and the VTR of the human c-H-*ras*1 gene. A synopsis of the reported regulatory sequences and nuclear factors, apart from the promoter region, essential for H-*ras* expression. Negative and positive regulatory elements of the first intron and the alternative exon are also shown. Exons, rectangles; coding sequences, filled rectangles; VTR, cross-hatched box; B, *Bam*HI cleavage site; S, *Sst*I cleavage site; dotted line, conserved intronic sequence. Nucleotide numbering starts from the *Bam*HI cleavage site.

information specified by the exon IV [5], as shown in Fig. 2. The predicted product of the alternative transcript is suggested to lack transforming potential, because of the absence of the exon IV-encoded C terminal sequence of the protein that is required for attachment of Ras protein to the membrane and for biological activity. Thus, alternative splicing normally operates to limit p21 levels. Point mutation of the donor splice site from this exon, can result in a 10-fold increase of full length p21 [44].

3. Overexpression of *ras* genes in human tumors

The *ras* genes are overexpressed in a wide variety of human tumors. Overexpression of *ras* oncogenes was examined by comparing the *ras* mRNA levels or the p21 levels in tumors, as compared with their respective normal tissue. Quantitative techniques were used, such as reverse transcription-polymerase chain reaction (RT-PCR) and RNA spot hybridization analysis, for detecting the overexpression of *ras* genes at the RNA level, and Western blotting and Immunohistochemistry, for indicating increased levels of the p21 protein. The results were often correlated with tumor histopathological features, tumor location and receptors for estrogens, progesterone and epidermal growth factor.

Table 1 summarizes the experimental results by indicating the tumor type where overexpression of *ras* genes was detected, the frequency of the overexpression, the detection methods followed, the member of the *ras* gene family implicated and the literature references. Where no particular *ras* gene is mentioned (referred as: *ras*), no discrimination between the *ras* family members nor their status (mutated or wild-type alleles) was performed.

3.1. Neuroblastomas

Overexpression of H-Ras protein was detected using Immunohistochemistry in 50–80% of cases [45,46].

Table 1
Incidence of overexpression of *ras* genes in human cancer

Tumor type	Frequency (%)	Method	*ras* genes	Reference
Neuro-blastoma	50–80	IHC	H-, *ras*	[45,46]
Head and neck	54	RTP, RHA	H-, K-	[47,48]
Esophagus	40	NBA	H-	[50,51]
Larynx	70	IHC	*ras*	[52]
	86	RTP	H-	[53]
	78	RTP	K-	[53]
	57	RTP	N-	[53]
Thyroid	85	IHC	*ras*	[54]
Lung	64–85	IHC	*ras*	[55,56]
Liver	60	IHC	*ras*	[57]
Small intestine	70	IHC	*ras*	[58]
Stomach	35	IHC, WB	K-, *ras*	[59,60]
Colon	31	RHA, IHC	H-, K-, *ras*	[61,62]
Breast	65–70	WB	*ras*	[63,64]
Bladder	39	RTP	H-	[70]
	58	RTP	K-, N-	[70]
Endometrium	18–95	IHC	*ras*	[72]
Ovary	45	WB, IHC	*ras*	[73,74]
Leukemias	39	RTP	H-	[75]
	61	RTP	K-	[75]
	67	RTP	N-	[75]

RTP, reverse transcription-PCR; RHA, RNA hybridization analysis; NBA, Northern blot analysis; WB, Western blotting; IHC, immunohistochemistry.

Some experimental results detected differences in the incidence of cases in which H-Ras protein was overexpressed when comparing the two major histological subtypes, neuroblastomas and ganglioneuroblastomas [45], whereas others did not [46]. Elevated p21 expression correlates with a favorable prognosis and early stages of the disease, thus suggesting that p21 levels detected by a simple and reproducible immunohistochemical procedure is of clinical importance in patients with this malignancy [45,46].

3.2. Head and neck tumors

Overexpression of the H-*ras* proto-oncogene is a frequent event in squamous-cell carcinomas of the head and neck (54%), as determined by RT-PCR [47]. Significant elevation was also shown for K-*ras* oncogene expression in both premalignant and malignant tumors [48]. It is suggested that alterations of *ras* genes are an early event in the development of head and neck carcinomas [49] and that H-*ras* gene overexpression is associated with a favourable prognosis [47].

3.3. Esophageal cancer

H-*ras* gene overexpression was detected in 40% of tumor specimens and in a significantly lower percentage of nondysplastic or dysplastic Barret's mucosa [50,51] using Northern blot analysis. It is suggested that *ras* genes are implicated in the progression of Barret's epithelium to invasive cancer and that H-*ras* expression provides a helpful marker for progression from dysplastic lesion to carcinoma.

3.4. Laryngeal tumors

Overexpression of Ras p21 protein was immunohistochemically detected in 70% of the laryngeal tumors tested [52]. Using RT-PCR, H-*ras* was overexpressed in 86%, K-*ras* in 78% and N-*ras* in 57% of cases [53]. No correlation between p21 expression and tumor location, stage, grade, epidermal growth factor receptor levels and amplification of *ras* genes was observed.

3.5. Thyroid neoplasms

Both papillary and follicular carcinomas showed consistently higher Ras p21 levels than the adjacent normal tissue in 85% of cases, using immunohistochemical studies [54]. Some adenomas (30%), also exhibited higher staining intensity than normal tissue, thus suggesting that elevated *ras* expression is implicated in the development of thyroid adenomas and their conversion to carcinomas [54].

3.6. Lung tumors

Ras p21 oncoprotein was overexpressed in 64–85% of non-small cell lung tumors, immunohistochemically tested [55,56]. Elevated p21 was detected in 56–80% of squamous cell carcinomas and in 68–90% of adenocarcinomas [55,56]. Ras p21 overexpresion was correlated with shorter survival of the patients [55] and is considered to be a late event in the development of lung cancer [56].

3.7. Hepatocellular carcinomas

Human hepatocellular carcinomas showed enhanced Ras p21 expression, in 60% of cases, as indicated by immunohistochemistry [57]. Little information concerning correlation of *ras* overexpression with clinicopathological parameters was provided.

3.8. Small intestinal tumors

Elevated Ras p21 expression was shown in 70% of human small intestinal tumors tested by immunohistochemistry, as compared with the adjacent normal tissue [58].

3.9. Gastric lesions

Overexpression of common *ras* and K-*ras* antigens was detected in gastric lesions by immunohistochemical and Western blot analysis [59,60]. Both H-*ras* and K-*ras* overexpression correlated with advance cancer stage, metastatic capacity, tumor invasion and poor prognosis of the disease [59,60].

3.10. Colorectal carcinomas

Study of H-*ras* and K-*ras* genes expression in premalignant polyps and malignant tumors of the colorectum, showed a significant elevation in both premalignant and malignant tissues, as compared with their respective normal mucosa, thus suggesting activation of the *ras* gene family in early stages of the development of colorectal carcinomas [61]. Immunohistochemical examination of the *ras* p21 expression, showed that 31% of cases were positively stained [62]. Overexpression of the *ras* genes was correlated with lymph node metastasis, the depth of invasion and with poor survival of the patients [62].

3.11. Breast tumors

Levels of the Ras p21 proteins higher than in normal breast tissues were found in 65–70% of cases [63,64] using Western blotting and overexpression of *ras* genes was shown at the RNA level as well [65]. No significant

correlation was seen between p21 level and the available clinical parameters [63,64], however, co-expression of oncogenes functioned as a prognostic correlate for recurrence and survival [64,66].

Although chemically induced neoplastic transformation correlated with alterations in H-*ras* gene, including loss of one allele and mutation of the remaining in codons 12 and 61 [67,68] in experimental models, *ras* mutations are infrequent in breast cancer patients. Therefore, we conclude that the Ras pathway contributes in the development of human breast cancer mainly by alterations in the expression levels of the gene and aberrations upstream or downstream of Ras or in Ras-related proteins [69].

3.12. Bladder tumors

H-*ras* transcripts were overexpressed in 39% of specimens, consisting of paired bladder tumor-adjacent normal tissue, while both the K-*ras* and the N-*ras* were detected in 58% of total specimens each. An RT-PCR technique was employed and each tumor specimen had a unique pattern of overexpression for the three *ras* genes [70]. Ras p21 overexpression is considered to be an early event in bladder neoplasias and is correlated with a great risk for developing invasive carcinoma from premalignant dysplasia and carcinoma in situ of the bladder [71].

3.13. Endometrial tumors

Activation of *ras* oncogenes has been implicated in human endometrial carcinomas using immunohistochemical methods in 95% of grade 2 and 3 adenocarcinomas studied, whereas only 18% of grade 1 cases were positive for *ras* peptides [72]. Thus, it is suggested that overexpression of the *ras* genes is a late event in carcinogenesis of the human endometrium.

3.14. Ovarian lesions

The enhancement of expression of *ras* genes is a late event in tumorigenesis of the ovary, with higher frequency in malignant tumors, than in normal, cystic ovaries and benign tumors [73]. Moreover, higher levels of the p21 protein in ovarian tumors was immunohistochemically found in 45% of cases [74]. The enhancement of p21 protein is associated with acquisition of metastatic potential [73] and simultaneous overexpression of the p21 and the c-*myc* protein p185 is correlated with shorter disease free and overall survival [74].

3.15. Leukemias

Elevated expression of the *ras* genes was found in 39, 61 and 67% of specimens for the H-*ras*, K-*ras* and N-*ras* genes respectively [75]. Moreover, it was shown that N-*ras* is overexpressed in all acute lymphoblastic leukemia cases, but in only half of the chronic lymphocytic leukemia cases. However, the number of the cases tested was limited and definite conclusions relating overexpression of the *ras* genes with particular clinicopathological parameters could not be drawn.

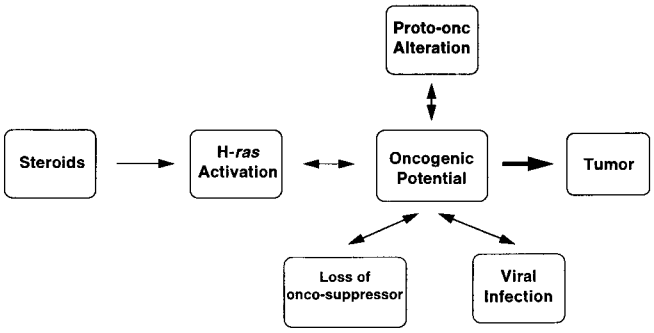

Fig. 3. Model for interaction of the H-*ras* gene and steroid hormones in the development of endometrial and ovarian cancer. Increased hormonally activated steroid receptor levels result in H-*ras* gene overexpression and in an increased oncogenic potential of the epithelial cells. Thus, the cells have a predisposition to develop neoplastic abnormalities in addition to a second tumorigenic event (proto-oncogene alteration, loss of an onco-suppressor, viral infection).

4. Involvement of the H-*ras* proto-oncogene and steroid hormones in gynecological cancer

Steroid hormones act as tumor promoters. The molecular mechanisms by which steroids are implicated in tumor promotion are not well defined, however, transcriptional activation of proto-oncogenes is proposed as one such mechanism and proto-oncogenes are theoretically well-suited for this role, as they participate in multiple signal transduction pathways and control gene expression in a cascade mechanism [76,77]. Moreover, elevated binding of the glucocorticoid and estrogen receptors in the corresponding regulatory elements of the H-*ras* proto-oncogene in human endometrial and ovarian tumors, as compared with the adjacent normal tissue, was observed in more than 90% of cases [78].

The human endometrium and ovary are major targets for action of glucocorticoids and sex hormones. This may result in an increased amount of ligand activated steroid receptors that bind to the H-*ras* DNA, resulting in an increased oncogenic potential. Thus, it is suggested [77] that endometrial and ovarian epithelial cells have a predisposition, due to H-*ras* hormonally-induced activation, to develop neoplastic abnormalities in addition to a second tumorigenic event, e.g. viral infection, mutational activation of a proto-oncogene or loss of an onco-suppressor gene (Fig. 3).

In addition, deregulated expression of the *ras* oncogene is associated with tumorigenic transformation of mammary cells through alterations in estradiol biotransformation [79,80]. The relative extend of estradiol metabolism leading to the formation of 16 alpha-hydroxyestrone increase in both normal H-*ras* proto-oncogene and mutant H-*ras* gene transfectant mammary epithelial cells and acquisition of anchorage-independent growth precede the emergence of a tumorigenic phenotype [79,80].

5. VTR rare alleles of the H-*ras* gene and ovarian cancer risk

Women who carry a mutation in the *BRCA*1 gene have a great risk of developing breast and ovarian cancer, but the penetrance of *BRCA*1 varies, thus suggesting that additional genetic and non-genetic parameters participate in the tumorigenesis of these individuals [81]. H-*ras* was the first example of a modifying gene on the penetrance of an inherited cancer syndrome. Rare alleles of the H-*ras* variable number of tandem repeat (VNTR) locus duplicate the magnitude of ovarian cancer risk for *BRCA*1 carriers, but not the risk for developing breast cancer [82]. It was suggested that different H-*ras* VNTR alleles show differences in modulating gene transcription, that H-*ras* VNTR alleles are in linkage disequilibrium with another gene important in tumorigenesis, or that rare alleles provide a marker for genomic instability [82].

6. Conclusions and perspectives

ras genes have been the focus of intense research since 1982, when their transforming alleles were first identified in human tumors. Unveiling the role of *ras* oncogenes in neoplastic development should have a major impact in understanding the pathogenesis of human cancer.

The key role of the H-*ras* expression in normal cellular growth and in oncogenic transformation underscores the importance of understanding the regulation mechanisms of the gene. In this review we summarized experimental results concerning regulatory elements of the promoter sequences [23–29], of the introns [30–36] and the VTR sequence [6,7,37] and discussed alternative mechanisms of regulating H-*ras* expression, such as methylation status [41–43] and alternative splicing of the RNA transcripts [44].

Overexpression of *ras* genes is a common event in human tumors. In a number of cancers, *ras* overexpression may be considered to be an early genetic event, for example in neuroblastomas [45,46], head and neck [49], thyroid [54], colorectal [61] and bladder tumors [71], whereas in others it may be interpreted as a late event, for example in lung [56], gastric [59,60], endometrial [72] and ovarian lesions [73]. Elevated expression of the Ras p21 protein has prognostic significance and is correlated with clinicopathological parameters in a variety of cancers [45–50,55,59–64,71,74], thus providing a helpful tool in early prognosis and diagnosis of the disease.

The development of effective molecular strategies for therapy remains the aim of tumor biology. Current therapeutic strategies include ribozymes against mutant *ras* gene products, antisense strategies, inhibitors of Ras protein posttranslational modifications and Ras peptide vaccination.

Molecular biology applies the site-specific RNAse properties of ribozymes to gene therapy for cancer. Requirements for substrate recognition and cleavage are mapped and strategies to accomplish high efficiency delivery of the ribozyme to target neoplastic cells must be developed. By using anti-Ras ribozymes, it was possible to reverse the neoplastic phenotype in mutant H-*ras* expressing tumor cells with high efficiency [83,84].

The antisense strategy involves reduction of expression of a particular gene by introduction of a cDNA segment in antisense orientation, in order to bind the target mRNA and prevent its translation [85]. Critical to the success of such an antisense agent is its ability to enter living cells, to specifically bind the target mRNA and induce RNAse-H cleavage of the target RNA. Activated *ras* genes, both by mutation or overexpression, are a common target of these therapeutical trials in cell-free and in vitro systems [86,87]. Clinical trials are now in progress to evaluate the therapeutic potential of antisense oligonucleotides targeting a variety of genes, e.g. H-*ras*, *myc*, p53, in tissue culture experiments and in vivo studies.

Furthermore, inhibitors of the Ras protein post-translational modifications are used as anticancer strategy. Farnesylation of the CAAX motif of Ras protein is essential for the subcellular localization of Ras to the plasma membrane and is critical to Ras cell-transforming activity. Inhibitors of farnesyltransferase have therefore been developed as a potential cancer therapeutic [88] and inhibition of farnesylation is shown to induce regression of carcinomas in transgenic animals [89].

Finally, Ras peptide vaccination is a recently developing molecular strategy for cancer therapy. Mutant Ras peptides are a candidate vaccine for specific immunotherapy in cancer patients. When vaccinated with a synthetic Ras peptide representing the *ras* mutation in their tumors, a transient Ras-specific T-cell response was induced in some of the patients treated. Ras peptide vaccination was proved to be effective in 40% of patients with pancreatic cancer [90,91]. However, pep-

tide vaccination, like all other gene therapy strategies previously mentioned, requires considerable development before useful anti-cancer agents can emerge [92].

Reviewers

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