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Ras and Ras mutations in cancer

Review Article

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Abstract: Ras genes are pre-eminent genes that are frequently linked with cancer biology. The functional loss of ras protein caused by various point mutations within the gene, is established as a prognostic factor for the genesis of a constitutively active Ras-MAPK pathway leading to cancer. Ras signaling circuit follows a complex pathway, which connects many signaling molecules and cells. Several strategies have come up for targeting mutant ras proteins for cancer therapy, however, the clinical benefits remain insignificant. Targeting the Ras-MAPK pathway is extremely complicated due its intricate networks involving several upstream and downstream regulators. Blocking oncogenic Ras is still in latent stage and requires alternative approaches to screen the genes involved in Ras transformation. Understanding the mechanism of Ras induced tumorigenesis in diverse cancers and signaling networks will open a path for drug development and other therapeutic approaches.

Keywords: *Ras • Cancer • Signaling • MAPK pathway • Oncogene*

1. Background history

Ras belongs to the family of small G proteins with intrinsic GTPases activity that governs various cellular signal transduction pathways. Activation of Ras GTPases results in a series of downstream signaling cascades, which initiate cell growth, differentiation, proliferation and cell survival [1]. Nascent Ras traffics from cytosol to plasma membrane, where it resides and communicates external signals to the nucleus. Certain point mutations within the Ras gene lock the protein into a constitutively active state, which leads to aberrant cell signaling even in the absence of external signals; such a dysregulated Ras signaling imminently leads to cancer instigation.

H-RAS and K-RAS genes were identified first and were ascertained for its tumerogenic potency. The existence of these 2 genes were discovered in Harvey and Kirsten sarcoma viruses, which infects rats as published by Jennifer Harvey [2] and Werner Kirsten [3]. The first study on the transforming traits of Ras genes were reported in early 1980s by G.M Cooper [4], Barbacid and Stuart [5] and by Robert Weinberg [6]. The years following 1982 validated an intensive research on Ras biology trying to decode its theatrical role in cancer progression. Further research subsequently identified a third human Ras gene and was designated N-ras, since it was reported first in human neuroblastoma cells. Altogether, the human genome incorporates 3 Ras genes and translates 4 protein products inspite of having 36 members in the Ras subfamily [7].

2. Gene location and classification

2.1 H-ras

H-RAS gene (named after Harvey) is localized to the short arm (p) of chromosome 11 at position 5 (Table 1). The gene spans 6.5 kb of human genome and contains 6 exons [8] and yields 2 alternate splice variants. Splicing at exon 2 and 5 generates p21 H-ras, the pre-

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Gene	Abbreviation	OMIM	Cytogenetic location	Common mutations	Reference
H-ras	Harvey rat sarcoma	190020	11p15.5	G12V, G12S, G12A, G13D, Q 61R	[122,123]
K-ras	Kirsten rat sarcoma	190070	12p12.1	G12D, G12S,G12R,G12A,G13D,G12V,G12C	[124,125]
N-ras	Neuroblastoma Ras	164790	1p13.2	G12D, Q61K	[126,127]
M-ras	Muscle Ras	608435	3q22.3	G22V, Q71L	[128]
R-ras	Related Ras	165090	19q13.33	-	[129]

Table 1. Classification of ras oncogenes.

eminent form, while splicing at exon 4 and 5 within intron D exon (IDX) results in the production of a rare p19- H-ras transcript [9], which is considered as a negative regulator of p21 H-ras [10]. The most common mutational hotspots in H-ras gene are confined to codon 12, 13 and 61. The mutations in exon 1 and 2 of H-ras gene have been reported in wide range of human cancers [11].

2.2 K-ras

K-RAS gene (named after Kristen) is located on the p arm of chromosome 12 at position 12.1 (Table 1).This gene consists of 6 exons and has 2 alternate spice variants namely K-ras 4A and K-ras4B. The K-ras 4A isoform includes all the coding regions in the mRNA transcript, while K-ras 4B excludes exon 6. Activating K-ras mutations are frequently reported in non-small-cell lung carcinoma (NSCLC), colorectal and pancreatic carcinomas [12,13]. These mutations are confined on exon 1 fragment of K-RAS gene and are marked by the substitution of glycine by aspartate/ valine at codon 12 and aspartate at codon 13 [14-16]. Most of the K-ras mutations detected in diverse tumors involve somatic mutations with a low incidence of germ line mutation.

2.3 N-ras

This gene gets its name from neuroblastoma cells in which they were reported initially. The cytogenetic location of N-RAS gene (Table 1) is on chromosome 1 at position 13.2. Mutations in N-RAS genes are generally somatic and not inherited. Overlapping mutation of N-ras with BRAF gene is consistent in cutaneous melanoma and proceeds to melanoma metastasis. Such mutations are also reported in acute lymphoblastic leukemia with higher incidence of N-ras codon 61 and 13 mutations. Mutations in N-RAS gene is also reported in Noonans syndrome with high frequency of T50I and G60E point mutations [17].

2.4 M-ras

M-ras has close resemblance to K-ras [18] with high degree of point mutations, G22V and Q71L (Table 1) M-ras gene is known to transform NIH3T3 cells and partially activates MAPK signal transduction pathway. The interaction of M-ras with other ras effectors is considerably poor as demonstrated in yeast two-hybrid system. These small GTPase proteins are also shown to involve in reorganization of actin cytoskeleton [19].

2.5 R-ras

R-ras are small GTPases, identical to the other ras proteins but possess less transformation efficiency (Table 1). Mutant R-ras do not display significant deviation in the expression pattern as observed in NIH3T3 cells, but forms colonies in soft agar and also develops tumor in nude mice. The mechanism of malignant transformation of R-ras does not have any correlation with other ras proteins and stimulates PI3-K/Akt pathway and not MAPK pathway [20]. R-ras also induces the activation of integrins *via* PI3-K/Akt pathway [21].

3. Constitutively active ras and cancer

Constitutive active Ras-MAPK pathway (Figure 1) is reported in a range of cancerous cells and tissues, in which defects in H-ras, K-ras and N-ras are frequently detected [22].

3.1 Ras-MAPK pathway

Ras is a member of the small G protein family that shuffle between GTP bound active and GDP bound inactive states [23]. Ras is a key regulator of MAPK pathway (Figure 1). The activation of MAPK pathway involves the binding of growth factors to protein tyrosine kinase receptors, which dimerize and in turn cross-phosphorylates tyrosine residues in the cytosolic domains. Phosphorylation of tyrosine recruits son of sevenless (SOS), which docks to the phosphotyrosine *via* Grb2 adaptor proteins. SOS also binds to plasma membrane and acts as Guanidine nucleotide exchange factor (GEF) for ras protein. The intrinsic GTPase activity of ras protein

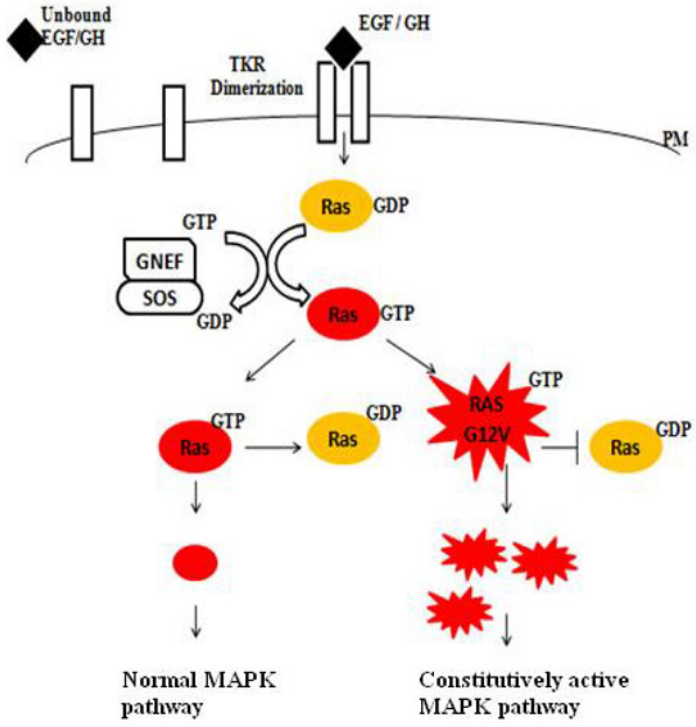

Figure 1. Normal and mutant Ras circuit connecting MAPK pathway. Upon receiving the external signals (Epidermal growth factor (EGF)/Growth factor (GH)), EGFR dimerizes and activates the tyrosine kinase activity by autophosphorylation of tyrosine residues. Docking protein, GRB2 associates with Son of seven (SOS) and facilities the removal of GDP from Ras. Further, Ras binds with GTP and phosphorylates Raf, MEK and ERK. Point mutations (G12V) in exon 1 of RAS gene locks the protein in its active state resulting in constitutive activation of MAPK pathway.

is stimulated by GAPs. Consequently the activated ras protein recruits Raf1 kinase, which phosphorylates raf1 leading to downstream signaling. Phosphorylated Raf1 (a MAP3K) in turn phosphorylates and activates MEK, a duel specific tyrosine/threonine kinase, (a MAP2K). MEK further phosphorylates and activates Erk1 and Erk2 (MAPKs), which translocates into the nucleus where it is shown to phosphorylate ELK1 transcription factor leading to the transcription of immediate early response genes. c-Fos and c-Jun are the transcription factors which are transcribed immediately in the vicinity as an outcome of Ras-MAPK signaling [24].

3.2 Ras and programmed cell death

Induction of apoptosis is a therapeutic strategy to target Ras-activated human cancers. The signaling cascade that relates ras protein with apoptosis opens a path to check tumor progression. Oncogenic ras expression can bring about positive or negative effects on apoptosis based on the cell type and external stimuli. Ras protein safeguards the cells from undergoing apoptosis either by activation of PKB/Akt *via* PI3-kinase, or through activation of NF-κB [25].

3.3 Pro-apoptotic activity of H-ras

Oncogenic H-ras (G12V) is known for its pro-apoptotic activity. The pro-apoptotic activity of H-ras increases the susceptibility of human colorectal adenocarcinoma HT29 cells when treated with histone deacetylase inhibitor (HDACi) [26] and TSA-induced apoptosis in mouse embryo fibroblast 10T1/2 cells [27-29]. HDACi mediates the activation of caspase 3,-7,-8 and effectively accords for cell death. Treating the cells with serine protease inhibitor AEBSF-HCl resulted in reduced cell viability demonstrating the role of serine proteases in induced apoptosis. The caspase 3 plays a vital role in induced apoptosis in Ras transformed 10T1/2 cells. Oncogenic Ras also meditates caspase 3 dependent apoptosis *via* mitogen-activated protein kinase, extracellular signal-regulated kinase and p53 in rostral ventrolateral medulla (RVLM) which in turn increases sympathetic nerve activity in stroke-prone spontaneously hypertensive rats [30].

Other anticancer agents that are involved in hastening the pro-apoptotic activity of oncogenic ras are 5-fluorouracil [31], etoposide VP16 [32], cisplatin [33], lovastatin [34] and arsenic [35]. The apoptotic effect of

H-ras G12V requires concurrent activation of ERK and JNK MAPK pathways. The dependence of H-ras G12V on p53 gene expression is self-subsistent. H-rasG12V dependent apoptosis is effectively blocked by the activation of the Ras GTPase. Activated H-ras gene is also determined to rescue adenovirus E1A induced apoptosis in primary baby rat kidney (BRK) cells. The gene works in coordination with E1A to repress p53 mediated growth arrest [36,37].

3.4 Ras and Fas expression

The Fas gene is located on the q arm of chromosome 10 at position 24.1 and is also known as TNFRSF6. This gene is one of the most important members of the TNFR super family. The involvement of H-ras gene in Fas mediated apoptosis is also reported. The mechanism by which mutant H-ras gene interacts with Fas is by DNA methylation. Activating Ras mutations are shown to silence Fas-induced apoptosis by promoter methylation, thus blocking the expression of Fas [38,39]. Similar inactivation patterns were also reported in HECIA cell line transformed with K-ras oncogene [40]. The silencing of Fas gene is achieved by intermediate effector proteins known as Ras epigenetic silencing effectors (RESE) (Figure 2). On transformation of K-ras mutant in NIH3T3 cells, these RESEs recruits DNA methyltransferase (DNMT1) to Fas promoter sites which selectively methylates and blocks Fas gene expression. Further studies on RESE knockdown assays in NIH3T3 cells showed regular phenotypic expression of Fas gene.

3.5 Apoptotic activity of K-ras isoforms

K-ras 4A and K-ras 4B are 189 and 188 amino acid proteins known for their tumorigenic potential [41]. K-ras 4B plays a vital role in tumor invasion and metastasis [42,43] by matrix metalloprotease-2 expression and cell migration while K-ras 4A is known to possess a tumor suppressor effect. This action was effective against carcinogen induced murine colonic adenoma formation [44]. The ratio of K-ras isoforms in cancerous tissues is maintained in equilibrium in the cellular system.

Ras protein affects susceptibility to apoptosis while mutant Ras is known to inhibit apoptosis [45]. Some studies support the concept that mutant Ras induces apoptosis [46]. K-ras protects the cells from undergoing apoptosis *via* PKB/AKT pathway [47]. Activated PKB/AKT pathway causes the phosphorylation of Bad, a pro-apoptotic protein. Phosphorylated Bad fail to form complexes with Bcl2, consequently the intracellular levels of Bcl2 accumulates and forms homodimers.

K-ras gene, when mutated, works antagonistically with its pro-apoptotic activity and is reported to enhance the rate of apoptosis. The dual-specific nature of K-RAS gene signaling results in both pro and anti apoptotic cascade. The integrity of K-ras mutation and apoptosis is maintained by synergistic effect of K-RAS gene expression with other oncogenes such as p53 and

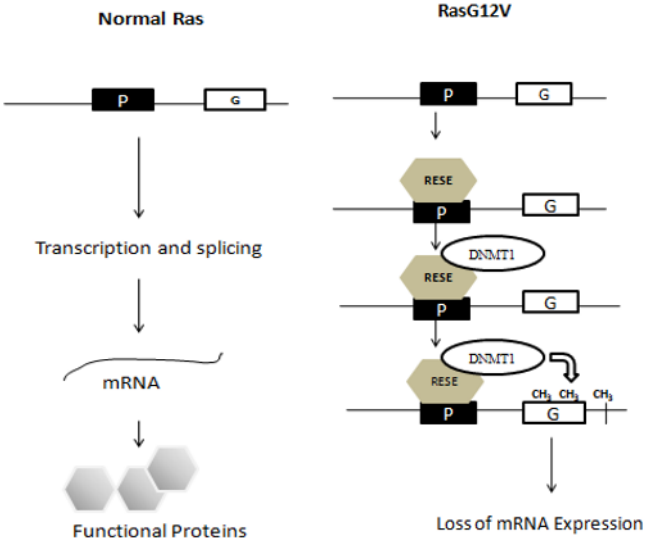

Figure 2. Role of Normal & mutant Ras in silencing Fas gene expression *via* RESE recruitment and DNA methylation. The mutant Ras (G12V) silences Fas expression *via* RESE. RESE recruits DNMT1 to Fas promoter sites, which methylates Fas gene and downregulates Fas mRNA expression. P – Promoter, G- Target gene, RESE- Ras epigenetic silencing effectors, DNMT1- DNA (cytosine-5)-methyltransferase 1, -CH_3 - Methyl group.

Rb [48]. It was also found that embryonic stem cells expressing mutant K-ras produces elevated $P19^{ARF}$, a tumor suppressor gene encoded by Ink4a-ARF locus [49]. $P19^{ARF}$ protein enforces the activation of p53 and renders the cells susceptible to DNA damage induced apoptosis [50].

K-ras activating mutations are highly reported in colon cancer [51] and are prevalent in lung and pancreatic cancers. K-ras 4A and K-ras 4B are found to co-express in colorectal cancer [52], with K-ras 4B having high potential to induce tumor compared to K-ras 4A isoform. K-ras 4B also plays a key role in cardiovascular homeostasis [53] and possess an anti-apoptotic effect [54] while K-ras 4A has a pro-apoptotic effect. The two splice forms undergo different post–translational modification, expression and function in a completely different manner [55]. Activated Ras is known to provide resistance to apoptosis in intestinal epithelial cells [56,57]. This is achieved by ras-induced down regulation of Bak, an effective regulator of apoptosis in intestinal epithelial cells [58,59]. Ectopic expression of Bak restored the normal mechanism and suppressed tumorigenicity [60,61].

3.6 Ras trafficking

The C-terminus end of Ras protein is essential for membrane targeting. The protein is trafficked to the plasma membrane following CAAX processing (Figure 3). The targeting involving Ras GTPase requires an additional second signal. This is achieved by palmitoylation of Ras GTPase at the N-terminus end of the α subunit [62,63]. The trafficking of nascent Ras occurs by two different pathways namely anterograde and reterograde pathway. Nascent Ras proteins are synthesized in the cytosol and gradually the processed proteins traffic from cytosol to endomembrane and finally to plasma membrane. Golgi apparatus serves as an intermediate platform for Ras trafficking.

CAAX processing is essential for producing a fully mature and functional protein (Figure 3). The CAAX sequence at the c-terminus of ras undergoes 3 step-processing events, which involves the addition of a farnesyl group to the cysteine residue by farnesyl transferase (FTase), removal of -AAX by proteolysis followed by carboxyl methylation [64], which takes place in endoplasmic reticulum [65]. H-ras, N-ras and K-ras 4A are further processed and modified by palmitoylation in the Golgi apparatus [66] while K-ras 4B doesn't require any further modification. K-ras isoforms differ from each other due to the different post-translational modification. K-ras 4A is palmitoylated at additional cysteine residue, while K-ras 4B contains a polybasic domain, essential for plasma membrane localization [67].

3.7 Nuclear localization of Ras

Ras proteins are predominantly localized in the plasma membrane and cytosol. Nuclear transport of Ras proteins (Figure 4) requires interaction with other GTPase known as Ran and other nuclear targeting proteins [68]. Immunofluorescence studies have described the localization of activated H-ras in nucleus. Consistent with the report it was shown that farnesylation of Ras is essential for nuclear targeting and transport. Inhibition of this CAAX processing step prevented the protein to translocate into the nucleus [69]. Activated H-ras is also found to complex with nuclear carrier protein known as NTF2 involved in nuclear import and export [70].

Scientists have also studied the localization of K-ras 4B isoform in the nucleoli of normal and transformed fibroblast cells. It was proposed that K-ras 4B isoform formed complex with nucleolin to co-localize in to the nucleus [71]. This study also reported the absence

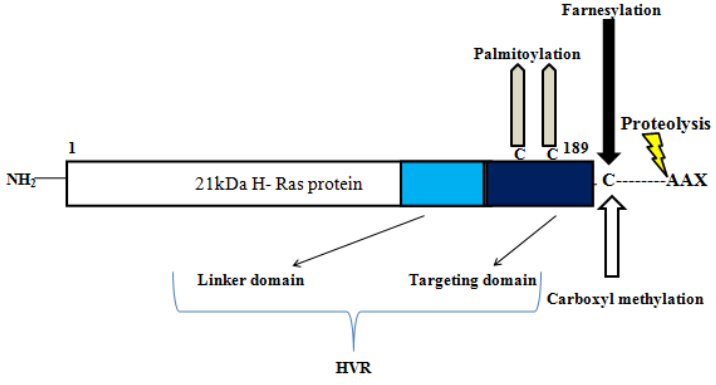

Figure 3. Protein structure of 21 kDa H-ras with its hypervariable region (HVR) and CAAX motif. HVR is divided in linker domain and membrane targeting domain (MTD). The MTD consists of C terminal CAAX motif, cysteine palmitoylation sites, which are common in H-ras, N-ras and K-ras4A protein while K-ras4B is rich in polybasic amino acids in the membrane-targeting domain.

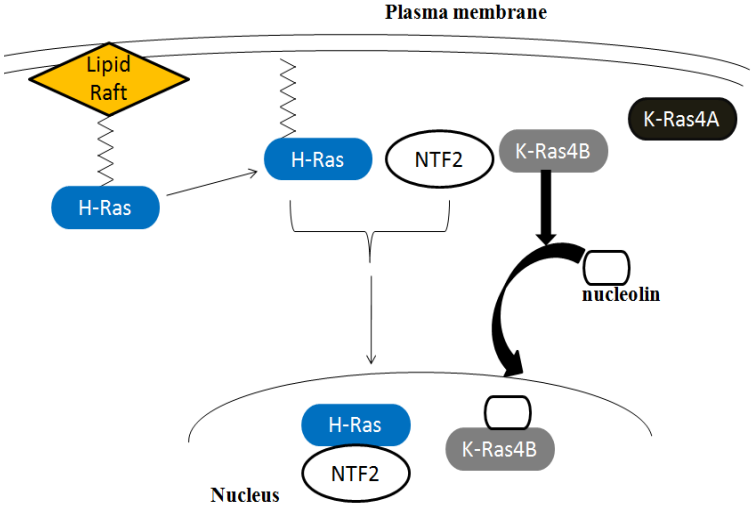

Figure 4. Nuclear localization of H-ras and K-ras 4B isoform. H-ras forms complex with NTF2 to translocate into the nucleus while K-ras 4B interacts with nucleolin to co-localize into the nucleus. K-ras4A is not shown to be involved in nuclear localization.

of H-ras and K-ras 4A expression in nucleus, also concerning the fact that inhibition of K-ras 4B-nucleolin complex prevented the transport of K-ras 4B into the nucleus. Nuclear expression of K-ras 4B was also reported in fibroblasts and mesangial cells but the expression of K-ras 4B reported by Birchenall-roberts *et al* was ruled out in this study due to the cross contamination of antibody reactivity [72].

3.8 Ras and Lipid Raft

Ras proteins essentially H-ras, K-ras 4A and N-ras undergo palmitoylation at the C-terminus end, which is required for membrane relocalization. H-ras and K-ras 4A are further translocated from Golgi to the cholesterol rich lipid rafts *via* a classical secretory pathway. Lipid rafts are microdomains, which comprises of cholesterol packed sphingolipids and glycosphingolipids [73]. Within the lipid rafts, H-ras protein exists in equilibrium, which is maintained by the hypervariable region linker domain. GTP loading shifts the equilibrium and leads to the detachment of the H-ras from lipid raft and subsequent dwelling within the plasma membrane [74,75]. K-ras 4B isoform fail to attach to the lipid rafts due to the modifications in the hypervariable region. It follows a lipid raft independent route to the plasma membrane.

3.9 Ras and NMD pathway

Nonsense mediated mRNA decay pathway (NDM) is popularly known as a quality control pathway due to its ability to degrade aberrantly spliced mRNA transcripts [76-78].The aberrantly splice transcripts contain a premature translation stop codons which are recognized by the proteins involves in the NMD pathway and are subsequently degraded. Involvement of H-ras gene is reported in NMD pathway. When the gene is not mutated, H-ras gene produces an additional splice variant different from the functional H-ras transcript, which is sensed and degraded in the cytosol [79]. Further studies discovered that synthesis of H-ras NMD splice variant requires p53 gene expression, when induced with camptothecin, a DNA topisomerase 1 inhibitor known to induce genotoxic stress. The transcription of this alternate spice variant though not productive could provide effective measures to eliminate oncogenic H-ras induced malignancies.

3.10 Ras and HDAC inhibitors

Ras-MAPK pathway effectively dictates chromatin organization and nuclear integrity. HDAC inhibitors are popular strategies of cancer therapeutics. HDAC treatment is known to alter the expression of Ras oncogenes, thus enhancing the efficiency of cancerous cells to undergo anoikis. It was first reported that ras-transformed rat epithelial cells undergo apoptosis when treated with sodium butyrate [80]. This HDAC treatment resulted in the inhibition of farnesylated ras protein with consistent reduction of Erk1 and Erk2 protein levels. Sodium butyrate thus achieves a prophylactic measures in tumor cells by interfering in the normal regulation of signaling pathways.

Ras signaling cascade is also known to translocate HDAC type 4 into the nucleus of primary neonatal cardiac myocytes. The nuclear transport of HDAC was achieved by H-ras activation by Epac, a GEF for ras family of GTPase [81]. Activated H-ras serves as a mediator for Epac induced cardiac myocyte hypertrophy *via* a signaling cascade involving Phosholipase C/IP3R

and an elevated intracellular calcium levels. Similar studies were done in C2C12 myoblast cells and results showed that transformation with oncogenic ras led to the increased localization of HDAC-4 in nucleus [82].

Certain other HDAC inhibitors like apicidin are known to reduce tumor invasiveness by epigenetic silencing. H-ras gene expression has a tremendous effect on the invasiveness in MCF10A breast epithelial cells. The gene dictates the secretion of MMP-2 [83]. The use of apicidin is manifested to reverse the phenotype and restore the biological rhythm [84]. The MMP-2 secretion in apicidin treated MCF10A cells showed drastic down regulation of MMP-2 thus exhibiting an anti-invasive effect, giving way for potential implementation of HDAC inhibitors for treating early metastatic tumors.

3.11 Ras and DNA methylation

Ras-MAPK pathway is major signaling pathway, which controls various epigenetic modifications in several human cancers and other syndromes. Oncogenic Ras is involved in DNA methylation of the promoter sequence of various tissue specific genes. It was first reported that DNA methylation in an adrenocorrtical tumor cell line Y1 was under the control of oncogenic H-ras expression. Y1 cells transformed with oncogenic H-ras gene resulted in hypermethylation in the CpG islands [85] and recruitment of DNMT1 in Y1 cells [86,87].

Researchers have identified that DNA hypermethylation at CpG islands up-regulates many genes linked with cancer [88]. G to A base transition at codon 12 in K-ras gene results in hypermethylation in the promoter of O6-Methylguanine-DNA methyltransferase (MGMT) gene causing loss of expression. Activated Ras also triggers the expression of DNA methytransferase 1 expression *via* Ras-AP-1 signaling. The involvement of ras gene in DNA methylation was further confirmed by treating with certain Ras-MAPK pathway inhibitors which led to the demethylation of many genes in colon cancer cells [89] and other malignant hematological diseases [90]. Other studies on RASAL, a Ca^{2+} regulated GTPase-activating-like protein (GAP) showed its ability to inactivate Ras gene in human tumors [91]. In its native state RASAL, which normally functions by decoding the frequency of Ca^{2+} oscillation is effectively silenced by CpG methylation following mutant Ras expression.

3.12 Mutant Ras and replicative senescence

Cellular senescence is a process of aging, which prevents the cell from immortalization. Senescent cells display enlarged, irregular morphology with enhanced secretion of beta-galactosidase and is well characterized by the presence of SAHFs. Oncogenic Ras expressions in MEF cells induce senescence (Figure 5) by the increased accumulation of p53 and p161NK4a protein levels. The induction of senescence by oncogenic ras follows a negative feedback signaling network since aberrant expression of mutant ras protein classically leads to variety of tumors. Only in a small proportion of benign tumors, mutant ras triggers replicative senescence. This network terminates the oncogenic potential of Ras and kick starts the senescence specific genes. The mechanisms, which connect p53 and Rb in senescence, are through the loss of expression of neurofibromin NF-1, a Ras GTPase activating protein [92].

It was established that activation of $p19^{ARF}$-p53 tumor suppressor pathway is indispensable for ras induced senescence. Ras G12V mutation intensifies the rate of senescence in human fibroblasts *via* the elevated expression of p21 Cdk inhibitor and P16. H-ras G12V works in conjunction with other transcription factors like CCAAT/enhancer binding protein β [93,94]. C/EBP β a bzip transcription factor is an essential contributor for skin tumor progression and has a

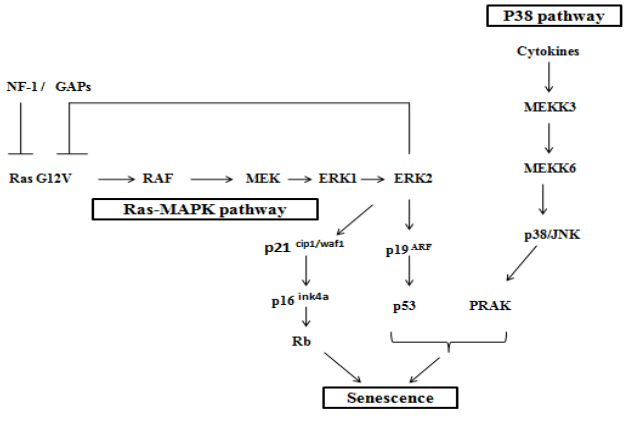

Figure 5. Various pathways controlled by oncogeneic Ras in inducing senescence.

significant role in oncogene induced senescence. Primary mouse fibroblast cells transformed with H-ras G12V is shown to accentuate senescence *via* C/EBP β while dominant negative form of C/EBP β restrains ras induced senescence in mouse NIH3T3 cells [95]. It was further substantiated that the expression of p16ink4a and $p19^{ARF}$ tumor suppressor genes were also lost in Ras transformed fibroblast cells [96]. Ectopic expression of $p19^{ARF}$ gene in NIH3T3 cells restored the levels of C/EBP β and thus marginally suppressed senescence. This study also stressed on the fact that ectopic expression of C/EBP β (Lap) in NIH3T3 cells inhibited ras mediated transformation with simultaneous activation of Fas receptors, consequently triggering apoptosis in these cells. Microarray data revealed two classical genes, which can serve as a novel prognostic marker for senescent cells which include topoisomerase IIα and HDAC9. The expressions of these 2 genes are drastically modulated in ras induced senescent cell.

A significant number of studies have suggested ROS actively participates in replicative senescence. Ras induced senescence (Figure 5) significantly elevates the mitochondrial ROS levels and mutant Ras fail to achieve senescence when fibroblast cells are supplemented with 1% oxygen [97]. Other studies have exemplified that mutant Ras triggers senescence in vascular smooth muscle cells in response to atherogenic stimuli [98]. Ras also functions in coordination with p38 regulated/activated protein kinase (PRAK) in programming the cells to senesce. This is achieved by the phosphorylation of tumor suppressor gene p53, ultimately allowing the cells to senesce [99].

The induction of oncogenic Ras mediated senescence can be rescued by inhibiting p21 cip1/Waf1 protein. It was witnessed that knockdown of p21 cip1/Waf1 using siRNA and other multiple miRNA rescued human mammary epithelial cells (HMECs) from senescence [100].

3.13 Targeting oncogenic Ras

The high incidence of oncogenic Ras in tumors attracts attention for anticancer therapy. The practical approach to achieve success is to obstruct the enzymes involved in post-translation modification of Ras proteins. The most common enzymes targeted are farnesyltransferase and geranylgeranyltransferase I, which are involved in prenylation of ras protein. Prenylation is a process of transferring the prenyl groups (15-carbon farnesyl or 20-carbon geranyl-geranyl) to the cysteine residues of the target protein at the C-teminal end (Figure 6). FTI's are prominent inhibitors of CAAX processing of ras protein and have emerged as a sensible and reliable anticancer drug [101-105]. Treatments with several FTI's in clinical trials have achieved productive outcome against activated Ras-MAPK pathway (Table 2). Though FTI's are effective against H-ras and N-ras, the drug has no response to cancers with activated K-ras mutation. The point that K-ras isoforms do not undergo CAAX processing (Figure 6) accounts for the ineffectiveness of FTI's tumor suppressing effect. Subsequently, anticancer therapy for K-ras induced malignancies is still a challenging task to accomplish. The use of geranylgeranyltransferase I inhibitors (GGTI's) is encouraged against K-ras4B isoform since

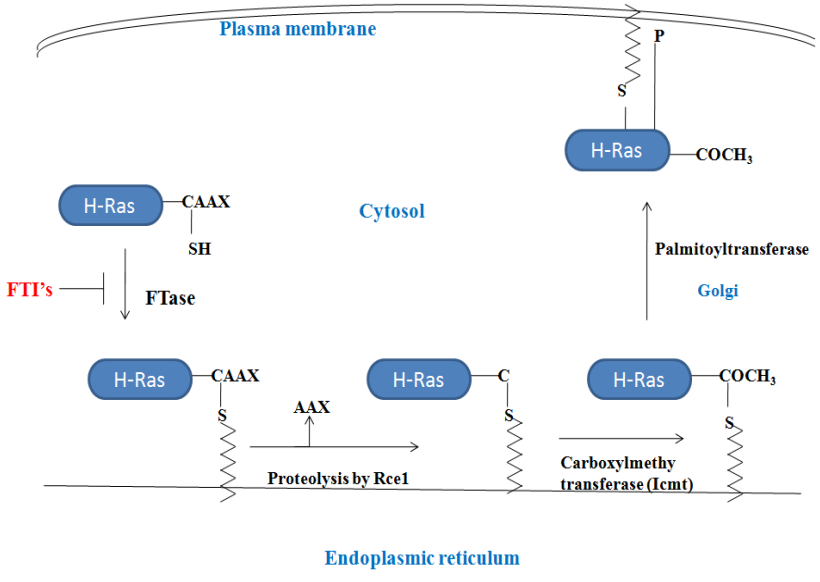

Figure 6. CAAX processing of H-ras protein. The diagram emphasizes on the region where FTase inhibitors act to check mutant Ras trafficking.

Drug	Phase	Single agent/combination	Results in experimental trials	Reference
FTI SCH66336 (Lonafarnib)	I	With temozolomide	Synergy. Tolerability: 150 mg - non- EIAEDs patients. 175 mg-EIAEDs patients.	[130]
	I	With gemcitabine	Endurable in patients with UTC. No synergy with gemcitabine.	[131]
	II	Single agent	Inactive in patients with TCC	[132]
	II	With paclitaxel	Tolerable up to 100 mg in sync with 175 mg/m^2 of paclitaxel in NSCLC patients	[133]
	II	With paclitaxel	Synergy. Anti-cancer activity in patients with taxane-refractory/resistant NSCLC.	[134]
FTI R115777 (Zarnestra,Tipifarnib)	II	Single agent	Active against metastatic breast cancer.	[135]
	II	Single agent	Endurable in patients with NSCLC.	[136]
	II	Single agent	Inhibition of FTase activity and phosphorylated ERK/ Akt pathways in patients with advanced melanoma	[137]
	II	Single agent	Tolerability in Patients with advanced solid malignancies	[138]
	II	Single agent	No anticancer activity in sensitive-relapse SCLC.	[139]
	III	Single agent	Endurable in refractory advanced colorectal cancer. No significant survival rate	[140]
	III	With gemcitabine	No synergistic effect in patients with APC. Endurable	[141]
FTI- BMS-214662	I	Single agent	Tolerable upto 118 mg/m^2 in patients with acute leukemia's and high-risk myelodysplastic syndromes.	[142]
	I	With paclitaxel	Synergy. Tolerable up to 160 mg/m/h, when in synergy with paclitaxel (80 mg/m^2/h) in patients with AST.	[143]
FTI- L-778,123	I	With radiotherapy	Acceptable toxicity (280 mg/m^2/day) in four NSCLC patients.	[144]
Gefitinib (EGFR-TKIs)	III	With gemcitabine and cisplatin	No synergy in patients with advanced NSCLC.	[145]
Erlotinib (EGFR-TKIs, OSI-774)	II	Single-agent (platinum-based chemotherapy)	Survival and accentuated tumor response in NSCLC patients	[146]
	III	With carboplatin and paclitaxel	No synergy in patients with NSCLC	[147]
	III	With mAb (bevacizumab)	Profound antitumor activity in NSCLC and soild tumors	[148]

Table 2. FTase and EGFR inhibitors of Ras-signaling pathway in clinical trials.

NSCLC – Non small cell lung cancer, SCLC - Small cell lung cancer, FT - Farnesyltransferase, TCC - Transitional cell carcinoma, EIAEDs - Enzyme-Inducing Anti-Epileptic Drugs, AST-Advanced soild tumors, APC – Advanced pancreatic cancer, UTC- Urothelial tract cancer.

the mutant form of K-ras 4B isoform is effectively geranylgeranylated. Anti-sense K-ras therapy is an alternate way to overcome this crisis [106]. The use of siRNA for silencing K-ras isoform is also shown to downregulate mutant K-ras mRNA levels [107,108].

Several inhibitor drugs are often rendered ineffective due the involvement of copious interrelated molecules and proteins in Ras-MAPK pathway, which develop strategies to evade drug treatments. To overcome this inefficiency, combinatorial drug therapies have been devised and are used in clinical trials. Several inhibitor combinations are employed to enhance the action of FTI's. Farnesyl thiosalicylic acid (FTS, Salirasib) is an inhibitor of Ras-mediated signaling that functions by dislodging Ras from the cell membrane [109,110] and the therapeutic mechanisms of action of FTS are in clear contrast to farnesyltransferase inhibitors (FTIs). The combination of FTS with glycolysis inhibitor had a synergistic effect in human glioblastoma multiforme (GBM) and pancreatic cells under study (Goldberg and kloog, Unpublished data). Other laboratories have reported combination of FTS with VEGF receptor inhibitor [111]. FTS with Bay 43-9006 has a profound anti-tumor activity against Raf GTPase and VEGF receptors [112]. This pair works in sync within lan1 neuroblastoma cells and blocks Myc oncogene activation.

Targeting epidermal growth factor receptor (EGFR) is an emerging field in clinical oncology. EGFR, a

member of the HER/ErbB family is a transmembrane growth factor receptor with tyrosine kinase activity. Activation of EGFR results in a series of phosphorylation events downstream Ras signaling pathway. Anti-EGFR monoclonal antibodies (mAbs) are frequently employed for cancer therapy. The most popular anti-EGFR mAbS are cetuximab and panitumumab [113,114]. Cetuximab is effective against metastatic colorectal cancer (mCRC), head and neck cancer while panitumumab is used for treating mCRC. K-ras mutations have strong correlation with EGFR, since the presence of activating K-ras mutations confer resistance to cetuximab and panitumumab in mCRC patients [115]. EGFR-TKIs (EGFR-tyrosine kinase inhibitors), erlotinib and gefitinib are the other proficient drugs in clinical trials with anti-tumor activity (Table 2). Recent studies on squamous cell anal carcinoma (SCAC) showed significantly less prevalence of EGFR and K-ras mutations in head and neck cancer [116] with cetuximab showing appreciable anticancer effect when administered in synergy with radiation.

An alternate strategy to treat Ras induced oncogenesis involves the use of competitive inhibitors against signal molecules and proteins downstream ras signaling cascade. The most potential targets are Raf kinase, mitogen-activated protein kinase (MEK) and phosphoinositide 3-kinase (P13K) [117]. P13K inhibitors inhibit the enzyme phosphoinsositide-3-kinases involved in P13K/AKT/mTOR pathway, an intracellular signaling cascade known to trigger apoptosis [118]. Certain other anthrapyrazolone inhibitors such as SP600125 restrain c-Jun N-terminal kinase [119]. Several medicinal plants with potential antineoplastic activity are also frequently administered to annihilate cancer. Recent researches have showed the use of carvacrol, a phenolic monotrepen from the Thyme plant with anti-Ras effect. Carvacrol exhibited significant growth arrest of 5RPT cells transformed with mutant H-ras and marginally inhibited N-ras transformed CO25 cell lines [120]. The study also highlighted the cytotoxic activity of carvacrol on H-ras transformed 5RPT cells and its ability to induce apoptosis in these cells, demonstrating the potential implementation of this carvacrol for treating Ras induced tumors. Scientists, working on molecular docking are also in a process for identification of binding sites within the ras protein for drug targeting. By nuclear magnetic resonance (NMR) studies, 25 small molecule compounds were shown to bind to the same location in ras protein concluding that the binding pocket in the protein was not inert and could be enlarged for administering next generation drugs [121].

4. Concluding remarks

Ras signaling circuit follows an intricate pathway, which revolves around various signaling molecules and connects several other pathways. Ras in cancer is a universal cell bio-marker, but the involvement of Ras in other syndromes is route to frame an alternate step towards Ras biology. Since 1982, several studies have identified the indispensable involvement of Ras in cancer, consequently leading to the genesis of diverse therapeutic approaches targeting oncogenic Ras. However, targeting Ras is still in its latent stage and needs to be precisely focused for developing newer anti-cancer drugs.

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