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MINIREVIEWS

RAS signaling pathways, mutations and their role in colorectal cancer

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Abstract

Two of the main cellular pathways in which the RAS protein operates are the mitogen-activated protein kinases (MAPK) and phosphoinositide-3 kinase (PI3K) pathways. In a normal cell, these are important in controlling several functions, such as cell growth and survival. It becomes self-evident that these events will be disrupted in a malignant cell with a deregulated MAPK or PI3K pathway. Mutations in genes involved in these pathways and interacting with RAS, as well as RAS itself will be discussed. The second part of this review concentrates on how crucial RAS signaling is in colorectal cancer progression, with references to treatment response and prognosis when RAS or other related mutations are present.

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Key words: Genes; RAS; Colorectal neoplasms; Therapeutics; Mitogen-activated protein kinase signaling system

Core tip: This review outlines clearly the normal function of the mitogen-activated protein kinases and phosphoinositide-3 kinase cascades, in which the RAS proto-oncogene operates physiologically. It also describes the mutations in these pathways that lead to colorectal cancer (CRC), as well as other mutations outside these cascades affecting RAS function and also leading to CRC. The prognostic value of each mutation is assessed and linked to response rates to available biological treatments. Monoclonal antibodies under development are also briefly discussed.

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MITOGEN-ACTIVATED PROTEIN KINASES SIGNALING CASCADE

Two of the main cellular pathways in which the RAS protein operates are the mitogen-activated protein kinases (MAPK) and phosphoinositide-3 kinase (PI3K) pathways. In a normal cell, these are important in controlling several functions, such as cell growth and survival[1,2]. The first step towards activating this pathway occurs when a ligand binds to a receptor tyrosine kinase (RTK). For example, a well known ligand is epidermal growth factor (EGF), whose receptor is EGFR. Before being able to bind EGFR, EGF must first be released from the cell surface membrane where it resides. This is achieved by means of the TACE/ADAM-17 enzyme, which is particularly capable of cleaving transforming growth factor-α and amphiregulin, two of the ligands belonging to the EGF family[3].

Following ligand binding, the receptor becomes dimerised and phosphorylated[4]. Next, a complex of proteins is established within the cell, with growth factor receptor-bound protein 2 (GRB2) becoming attached to the receptor, whilst being bound by son of sevenless

(SOS). Then the SOS protein, whilst still attached to GRB2, binds RAS as well. It should be noted here that there are several subtypes of the RAS protein, such as HRAS, NRAS and KRAS, but the most important one with regards to colorectal carcinogenesis is the latter, followed by NRAS[1].

After the attachment of SOS to RAS, SOS shows guanine nucleotide exchange factor activity. This means that SOS is capable of displacing guanosine diphosphate (GDP) molecules from RAS and thus allowing guanosine triphosphate (GTP) molecules to bind and activate it. Active GTP-RAS is able to recruit the RAF proteins (A-RAF, B-RAF and C-RAF) to the cell surface. The RAF proteins are normally bound to, and therefore inhibited by, the 14-3-3 proteins in the cytosol. However, after binding to GTP-RAS, the RAF proteins are released from the 14-3-3 proteins and are therefore activated; they pair up amongst them and form heterodimers, which are then capable of binding and activating the KSR1 enzyme[1].

The KSR1 enzyme is a relay hub connecting RAF heterodimers with the MEK protein. Hence, RAF proteins are able to phosphorylate and activate MEK, which in turn phosphorylates and activates ERK. ERK then enters the cell nucleus to activate a range of transcription factors, such as Jun and Fos[1]; these bind to the AP-1 DNA domain of the nucleus and transcribe genes involved in cell proliferation[5].

The above process is entirely normal in a healthy cell, and is terminated by means of RAS-GTPase activating (GAP) proteins. As their name suggests, these proteins activate GTPase enzymes found within RAS, which hydrolyse GTP to GDP and therefore switch RAS off[1].

MUTATIONS RELATIVE TO THE MAPK PATHWAY

RAS mutations

One of the most frequent ways in which the MAPK is set to overdrive is by a mutation in the RAS protein; mutations in the KRAS protein are found in about 40% of all colorectal cancer (CRC) cases, whereas *NRAS* mutations are less common, having a frequency of 5%. Both in KRAS and NRAS, the most typical mutations are found at codons 12, most of the times, 13 and 61 (the latter being rarely affected). These mutations are sometimes present in early adenomas and in cells with minimal potential to develop a malignancy. However, they are also thought to enhance the malignant behaviour of cells with advanced CRC; both *in vitro* and animal studies indicate that silencing these mutated codons leads to attenuation of the tumourigenic growth properties of the affected cells[6].

In molecular terms, mutations in these three KRAS/NRAS codons may lead to conformational changes so that the RAS-GAP protein cannot activate the inherent GTPase enzyme anymore. As a result, the GTP molecules are not hydrolysed and instead they maintain RAS continuously in its active state, thus causing protumorigenic effects by amplifying signaling in the MAPK pathway[7].

BRAF mutations

BRAF can also be mutated in the MAPK pathway, and this appears to happen in about 5%-10% of all colon cancer cases[6]. The commonest BRAF mutation amongst all cancers, including colorectal, is the V600E mutation. This occurs when adenine replaces thymine at nucleotide 1799. Consequently, glutamic acid (E) substitutes valine (V) at codon 600, hence the name of the mutation[8,9].

Two basic models were proposed to explain how CRCs arise, and *BRAF* mutations occur in both of them. The first model proposed by Fearon and Vogelstein in 1990 suggested that CRC is a result of multiple adenomatous lesions progressing to carcinomas, following several somatic and inherited gene alterations[10]. Apparently, this is what happens in the majority of the cases[11]. In this model, the mutation of adenomatous polyposis coli (APC) leading to initial formation of the polyps is of paramount importance[12].

The second model, true in approximately 15% of all CRC cases[6], holds that CRC is caused by mutation of mismatch repair (*MMR*) genes, which normally fix errors in DNA replication. Hence the mutations result in replicative errors not being corrected and therefore microsatellites (short DNA repetitions) start accumulating or become abnormally short, leading to microsatellite instability (MSI)[13] and colorectal carcinogenesis. Inactivation of *MMR* genes can be observed in the hereditary non-polyposis CRC (HNPCC) syndrome, but it usually occurs epigenetically; epigenetic inactivation most often involves the hypermethylation of MutL homolog 1 (*MLH1*), one of the *MMR* genes, and falls under a category of colorectal tumours called CpG island methylator phenotype (CIMP). CIMP tumours have a specific histological appearance, termed sessile serrated adenomas (SSAs). *BRAF* mutations are very frequent in SSAs, but not so frequent in HNPCC. Overall, they are mostly found amongst sporadic, high in MSI (MSI-H) colorectal tumours[6].

In any case, a *BRAF* mutation will lead to increased kinase activity and therefore increased downstream signaling in the MAPK cascade[6].

EGFR and other RTK mutations

EGFR (*HER-1*) gene amplifications or point mutations may cause an up-regulation of the receptor, thus increasing the probability of its activation by EGF binding and thus increasing signaling. However, such events are quite uncommon and appear in less than 5% of CRCs[14]. The human epidermal growth factor receptor 2 (HER-2)/neu receptor can also be overexpressed; though the evidence is inconsistent and ranges are anywhere between 0% and 83%, it is unlikely that this is a major determinant of colorectal tumorigenesis[15].

PI3K SIGNALING CASCADE

The other main pathway in which RAS is involved is the

PI3K pathway. This is a very complex pathway, therefore only some of its key elements will be mentioned here. Just like in the case of the MAPK pathway, various growth factors initially bind on receptor tyrosine kinases, leading to their dimerisation and autophosphorylation. The next stage involves PI3Ks. There are three different classes of PI3Ks, but the most important class in human cancer is IA. The regulatory subunit of this class, p85, attaches to phosphotyrosine residues and/or other adaptors found on the RTKs. As a result, p110, the catalytic subunit of the PI3Ks is disinhibited and phosphorylates PIP2 to PIP3[2]. RAS can also activate the pathway physiologically by directly binding p110[16]. Conversely, the tumour suppressor protein PTEN dephosphorylates PIP3 back to PIP2, thus terminating signaling[2].

Once PIP3 is formed, it recruits PDK1 and AKT kinases and brings them in close proximity. PDK1 phosphorylates AKT. Consequently, AKT becomes activated and generates several signals, the details of which are probably unrelated to this topic. These signals essentially contribute to cellular growth and evasion of apoptosis[2].

MUTATIONS RELATIVE TO THE PI3K PATHWAY

PIK3CA mutation

PIK3CA is the gene encoding for P110α. Mutation of RAS often coexists with mutations at exons 9 and 20 of *PIK3CA*[17]. It has been hypothesised that, when RAS is mutated, it can no longer bind the physiological form of P110α efficiently. This necessitates the mutation of *PIK3CA*, which apparently will encode for a truncated version of P110α, on which the mutant RAS will be able to bind effectively. The estimated frequency of *PIK3CA* mutations in CRC is 15%-25%, and these may lead to increased PI3K activity[6].

Phosphatase and tensin homolog mutation

Nonsense mutations and deletions in the phosphatase and tensin homolog (*PTEN*) gene makes the PTEN protein unable to convert PIP3 to PIP2, thus it can no longer act as an antagonist to PI3K signaling. This mutation is cardinal to the manifestation of Cowden syndrome, as it appears in 85% of all its cases. Cowden syndrome is an autosomal dominant disorder that predisposes to multiple cancers, including colorectal. Overall, it is estimated that *PTEN* is mutated in 10%-20% of all CRCs[6].

OTHER MUTATIONS AFFECTING RAS SIGNALING

Neurofibromin 1 mutations

The neurofibromin 1 (*NF1*) gene is responsible for causing the genetic disease neurofibromatosis type 1. *NF1* acts as a negative regulator of RAS because it transcribes neurofibromin, a GAP; as mentioned above, these proteins hydrolyse RAS-bound GTP to GDP, therefore inactivating RAS. It has been suggested that NF1 may play a role in colorectal carcinogenesis when mutated, because it can no longer inhibit RAS signaling effectively. Indeed some studies have found increased *NF1* mutations in malignant colorectal tissue[18,19], and in concurrence with *KRAS* mutations as well. Having said that, *NF1* mutations may also occur with wild type *KRAS*. In addition, one study found that the majority of *NF1* mutations were actually concurrent with *BRAF* mutations, especially in MSI-H tumours[18]. Paradoxically, a more recent *in vitro* study observed that the mitogen-activated protein kinase (MAPK) pathway signaling was upregulated in malignant colorectal cells with wild type *BRAF* and a knocked out *NF1* gene[20]. Therefore, the role of NF1 in human colorectal carcinogenesis remains largely controversial.

The inverse relationship between CRC and neurofibromatosis type 1 is also evident; rarely, children with homozygous deficiency of the *MLH1* gene, which leads to HNPCC, exhibit features of neurofibromatosis type 1[21].

RASSF mutations

The RASSF is a family of ten genes (*RASSF1-10*), members of which seem to act as tumour suppressors. An emerging body of evidence indicates that they can stimulate growth arrest and proapoptotic signals mediated by RAS. The exact way they achieve this is still unclear; there are suggestions that there is a domain in RAS which *RASSF* can bind, and indeed this holds true to date for *RASSF1*, *RASSF2*, *RASSF4* and *RASSF5*. *RASSF1A* is one of the most well studied members of the family[22], and it has been said that it either binds farnesylated KRAS directly[18,23] or it has to form a heterodimer with RASSF5 before is able to bind RAS[18,24].

Silencing of *RASSF1A* may occur when a specific sequence on the gene, the CpG island promoter region [*i.e.*, a region rich in cytosine (C) and guanine (G) nucleobases linked by phosphodiester (p) bonds] is methylated. This event is regarded to be a major contributor to early CRC development, ranging from 12% to 81% amongst different studies. Methylation can also affect *RASSF2* and *RASSF5* in the context of CRC. Nevertheless, exactly how they bring about malignancy is currently under investigation[22].

HOW IMPORTANT RAS SIGNALING IS FOR CRC

In order to evaluate the importance of RAS signaling, it is reasonable to examine how RAS and associated mutations behave in the clinical setting; whether they respond to current treatments, and how good the prognosis is when such mutations are evident.

Issues with KRAS

Screening for KRAS mutation is the only widely used and accepted prognostic tool to decide eligibility for monoclonal antibody therapy[25,26]. This is largely because, the only molecular treatment currently licensed to be used in clinical practice is anti-EGFR therapy[26]. Hence, the

identification of a *KRAS* mutation is used as a means of exclusion from anti-EGFR therapy. The rationale for this is that since the MAPK pathway signaling is upregulated by a constitutively active KRAS protein, it is worthless to try and block the EGFR since MAPK signaling is no longer dependent on the activation of the receptor[25].

However, the above notion, though probably true most of the times, is not universally accepted. Although mutant *KRAS*, especially the G12V mutation, is often associated with poor response to anti-EGFR agents[26], some studies have not found the same results. Several clinical studies observed that patients with a p.G13D (codon 13) mutation in KRAS actually responded to treatment with cetuximab, as they had increased progression-free and overall survival compared to those on best supportive care or chemotherapy alone. This is a conclusion not to be ignored, because p.G13D positive patients are often refused administration of cetuximab (based on the rationale described above), albeit they could potentially benefit from it. Hence, further prospective clinical trials should be performed to confirm these data, since the value of the KRAS p.G13D mutation as a negative predictive biomarker is still contradictory[27].

In addition, regarding metastatic CRC, there is also a question of whether the primary or the metastatic lesion should be analysed for genetic mutations. Some researchers postulate that there is no difference between the two, whereas others report significant variations. It is also argued that the genetic profile of the metastatic lesion is what matters most, because it is the metastasis that causes the bulk of the morbidity and mortality related to the disease. These hypotheses may again have ethical implications. In a hypothetical scenario, a patient has a genetic variation between his primary and metastatic lesions; the majority of his metastatic cells carry the wild type *KRAS* gene, but the primary lesion has mutant alleles. Based on the latter, he is wrongfully denied potentially beneficial anti-EGFR therapy, if indeed the metastasis is what's causing the major problem[26].

Finally, there is uncertainty regarding how many and which KRAS codons should be screened, as well as issues with cost-effectiveness[26].

Clinical status of other mutations

There are a great number of cases with wild type KRAS tumours which fail to respond to anti-EGFR therapy. This of course might happen because there are other mutations disrupting the MAPK pathway. These may include mutations in other regions of the KRAS gene which are not commonly tested. Indeed, the majority of clinical trials regarding *KRAS* mutations in CRC involved screening codons 12 and 13 only, whereas there are reported mutations in exons 3 and 4 as well[26]. NRAS also becomes mutated occasionally.

BRAF mutation is associated with very poor prognosis as it does not respond to anti-EGFR therapy. In fact, in a study performed by Di Nicolantonio *et al*[28], it was observed that none of the patients with a V600E *BRAF* mutation responded to either cetuximab or panitumumab. *BRAF* mutations are also mutually exclusive with KRAS mutations, *i.e.*, these two do not occur together[18,25-28]. This means that if mutant *KRAS* is identified in a patient, there is no point of screening for BRAF as well. It is rather more useful to screen for KRAS first, since some particular mutations, as already discussed, may validate the use of anti-EGFR therapy. This is not the case for BRAF, where there is no response to monoclonal antibodies whatsoever. Hence, as a prognostic biomarker, BRAF can only be used to indicate complete insensitivity to anti-EGFR agents.

Contrary to BRAF, PIK3CA and PTEN are not mutually exclusive to KRAS[29]. Loss of function of PTEN or mutation in *PIK3CA* is often associated with poorer response to cetuximab or panitumumab, as expected. However, there is no standard, reliable scoring system by which PTEN loss can be detected, thus making it an unsuitable prognostic biomarker. At the same time, the data regarding *PIK3CA* mutations is not uniform, as some studies report no overall difference in 5-year survival for patients with *PIK3CA* mutation, whilst others report positive response to cetuximab[29].

The future

Most patients responding well to current treatments are essentially those who only have EGFR upregulation. All the other mutations necessitate the discovery of agents that can block RAS signaling further down the pathway. Such a discovery will render any specific gene alteration irrelevant. Indeed, there has been a development of a RAF inhibitor, called PLX4032, which showed inhibition of RAF in melanoma, but had little success with CRC cells[30]. Similarly, AZD6244, a MEK inhibitor which recently entered phase 2 trials for CRC showed no significant advantage over chemotherapy[26].

CONCLUSION

It has recently been said that the MAPK and PI3K cascades are important to carcinogenesis and progression of CRC[25]. Apart from *KRAS* mutations, which are the main reason why anti-EGFR agents fail, several other genes related to RAS signaling also contribute to CRC manifestation and, some more than others, to anti-EGFR therapy insensitivity. If nothing else, *KRAS* and *BRAF* mutations can be used as negative biomarkers to identify patients who will not benefit from cetuximab and panitumumab. Nevertheless, as mentioned earlier, we do not know everything, as there are still several controversies in the data regarding the clinical status of some mutations, as in the case of p.G13D KRAS, PIK3CA and others. These indicate that there is a need to perform larger clinical trials that will minimise statistical error and will find out what exactly happens in these cases. In doing so, perhaps a deeper insight will be gained into the molecular mechanisms giving rise to CRC, thus allowing for pioneering pharmacological agents to be successfully developed.

REFERENCES

1 **Nandan MO**, Yang VW. An Update on the Biology of RAS/RAF Mutations in Colorectal Cancer. *Curr Colorectal Cancer Rep* 2011; **7**: 113-120 [PMID: 21625338 DOI: 10.1007/s11888-011-0086-1]

2 **Courtney KD**, Corcoran RB, Engelman JA. The PI3K pathway as drug target in human cancer. *J Clin Oncol* 2010; **28**: 1075-1083 [PMID: 20085938 DOI: 10.1200/JCO.2009.25.3641]

3 **Merchant NB**, Voskresensky I, Rogers CM, Lafleur B, Dempsey PJ, Graves-Deal R, Revetta F, Foutch AC, Rothenberg ML, Washington MK, Coffey RJ. TACE/ADAM-17: a component of the epidermal growth factor receptor axis and a promising therapeutic target in colorectal cancer. *Clin Cancer Res* 2008; **14**: 1182-1191 [PMID: 18281553 DOI: 10.1158/1078-0432.CCR-07-1216]

4 **Reuter CW**, Morgan MA, Bergmann L. Targeting the Ras signaling pathway: a rational, mechanism-based treatment for hematologic malignancies? *Blood* 2000; **96**: 1655-1669 [PMID: 10961860]

5 **Ubeda M**, Vallejo M, Habener JF. CHOP enhancement of gene transcription by interactions with Jun/Fos AP-1 complex proteins. *Mol Cell Biol* 1999; **19**: 7589-7599 [PMID: 10523647]

6 **Fearon ER**. Molecular genetics of colorectal cancer. *Annu Rev Pathol* 2011; **6**: 479-507 [PMID: 21090969 DOI: 10.1146/annurev-pathol-011110-130235]

7 **Mitin N**, Rossman KL, Der CJ. Signaling interplay in Ras superfamily function. *Curr Biol* 2005; **15**: R563-R574 [PMID: 16051167 DOI: 10.1016/j.cub.2005.07.010]

8 **Tan YH**, Liu Y, Eu KW, Ang PW, Li WQ, Salto-Tellez M, Iacopetta B, Soong R. Detection of BRAF V600E mutation by pyrosequencing. *Pathology* 2008; **40**: 295-298 [PMID: 18428050 DOI: 10.1080/00313020801911512]

9 **Dhomen N**, Marais R. New insight into BRAF mutations in cancer. *Curr Opin Genet Dev* 2007; **17**: 31-39 [PMID: 17208430 DOI: 10.1016/j.gde.2006.12.005]

10 **Fearon ER**, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990; **61**: 759-767 [PMID: 2188735 DOI: 10.1016/0092-8674(90)90186-I]

11 **Ballinger AB**, Anggiansah C. Colorectal cancer. *BMJ* 2007; **335**: 715-718 [PMID: 17916855 DOI: 10.1136/bmj.39321.527384.BE]

12 **Markowitz SD**, Bertagnolli MM. Molecular origins of cancer: Molecular basis of colorectal cancer. *N Engl J Med* 2009; **361**: 2449-2460 [PMID: 20018966 DOI: 10.1056/NEJMra0804588]

13 **Oki E**, Oda S, Maehara Y, Sugimachi K. Mutated gene-specific phenotypes of dinucleotide repeat instability in human colorectal carcinoma cell lines deficient in DNA mismatch repair. *Oncogene* 1999; **18**: 2143-2147 [PMID: 10321739 DOI: 10.1038/sj.onc.1202583]

14 **Heinemann V**, Stintzing S, Kirchner T, Boeck S, Jung A. Clinical relevance of EGFR- and KRAS-status in colorectal cancer patients treated with monoclonal antibodies directed against the EGFR. *Cancer Treat Rev* 2009; **35**: 262-271 [PMID: 19117687 DOI: 10.1016/j.ctrv.2008.11.005]

15 **Schuell B**, Gruenberger T, Scheithauer W, Zielinski Ch, Wrba F. HER 2/neu protein expression in colorectal cancer. *BMC Cancer* 2006; **6**: 123 [PMID: 16681853 DOI: 10.1186/1471-2407-6-123]

16 **Shaw RJ**, Cantley LC. Ras, PI(3)K and mTOR signalling controls tumour cell growth. *Nature* 2006; **441**: 424-430 [PMID: 16724053 DOI: 10.1038/nature04869]

17 **Baldus SE**, Schaefer KL, Engers R, Hartleb D, Stoecklein NH, Gabbert HE. Prevalence and heterogeneity of KRAS, BRAF, and PIK3CA mutations in primary colorectal adenocarcinomas and their corresponding metastases. *Clin Cancer Res* 2010; **16**: 790-799 [PMID: 20103678 DOI: 10.1158/1078-0432.CCR-09-2446]

18 **Ahlquist T**, Bottillo I, Danielsen SA, Meling GI, Rognum TO, Lind GE, Dallapiccola B, Lothe RA. RAS signaling in colorectal carcinomas through alteration of RAS, RAF, NF1, and/or RASSF1A. *Neoplasia* 2008; **10**: 680-686, 686 [PMID: 18592002]

19 **Cacev T**, Radosević S, Spaventi R, Pavelić K, Kapitanović S. NF1 gene loss of heterozygosity and expression analysis in sporadic colon cancer. *Gut* 2005; **54**: 1129-1135 [PMID: 15840687 DOI: 10.1136/gut.2004.053348]

20 **Ali MA**, Sjoblom T. Core Ras pathway signaling in human colorectal cancers revealed by isogenic modelling of NF1, KRAS and BRAF. *Eur J Cancer* 2012; **48**: 118 [DOI: 10.1016/S0959-8049(12)71162-0]

21 **Gallinger S**, Aronson M, Shayan K, Ratcliffe EM, Gerstle JT, Parkin PC, Rothenmund H, Croitoru M, Baumann E, Durie PR, Weksberg R, Pollett A, Riddell RH, Ngan BY, Cutz E, Lagarde AE, Chan HS. Gastrointestinal cancers and neurofibromatosis type 1 features in children with a germline homozygous MLH1 mutation. *Gastroenterology* 2004; **126**: 576-585 [PMID: 14762794 DOI: 10.1053/j.gastro.2003.11.008]

22 **Fernandes MS**, Carneiro F, Oliveira C, Seruca R. Colorectal cancer and RASSF family--a special emphasis on RASSF1A. *Int J Cancer* 2013; **132**: 251-258 [PMID: 22733432 DOI: 10.1002/ijc.27696]

23 **Donninger H**, Vos MD, Clark GJ. The RASSF1A tumor suppressor. *J Cell Sci* 2007; **120**: 3163-3172 [PMID: 17878233 DOI: 10.1242/jcs.010389]

24 **Ortiz-Vega S**, Khokhlatchev A, Nedwidek M, Zhang XF, Dammann R, Pfeifer GP, Avruch J. The putative tumor suppressor RASSF1A homodimerizes and heterodimerizes with the Ras-GTP binding protein Nore1. *Oncogene* 2002; **21**: 1381-1390 [PMID: 11857081 DOI: 10.1038/sj.onc.1205192]

25 **Rizzo S**, Bronte G, Fanale D, Corsini L, Silvestris N, Santini D, Gulotta G, Bazan V, Gebbia N, Fulfaro F, Russo A. Prognostic vs predictive molecular biomarkers in colorectal cancer: is KRAS and BRAF wild type status required for anti-EGFR therapy? *Cancer Treat Rev* 2010; **36** Suppl 3: S56-S61 [PMID: 21129611 DOI: 10.1016/S0305-7372(10)70021-9]

26 **Vakiani E**, Solit DB. KRAS and BRAF: drug targets and predictive biomarkers. *J Pathol* 2011; **223**: 219-229 [PMID: 21125676 DOI: 10.1002/path.2796]

27 **Yokota T**. Are KRAS/BRAF mutations potent prognostic and/or predictive biomarkers in colorectal cancers? *Anticancer Agents Med Chem* 2012; **12**: 163-171 [PMID: 22043994 DOI: 10.2174/187152012799014968]

28 **Di Nicolantonio F**, Martini M, Molinari F, Sartore-Bianchi A, Arena S, Saletti P, De Dosso S, Mazzucchelli L, Frattini M, Siena S, Bardelli A. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. *J Clin Oncol* 2008; **26**: 5705-5712 [PMID: 19001320 DOI: 10.1200/JCO.2008.18.0786]

29 **Asghar U**, Hawkes E, Cunningham D. Predictive and prognostic biomarkers for targeted therapy in metastatic colorectal cancer. *Clin Colorectal Cancer* 2010; **9**: 274-281 [PMID: 21208841 DOI: 10.3816/CCC.2010.n.040]

30 **Joseph EW**, Pratilas CA, Poulikakos PI, Tadi M, Wang W, Taylor BS, Halilovic E, Persaud Y, Xing F, Viale A, Tsai J, Chapman PB, Bollag G, Solit DB, Rosen N. The RAF inhibitor PLX4032 inhibits ERK signaling and tumor cell proliferation in a V600E BRAF-selective manner. *Proc Natl Acad Sci U S A* 2010; **107**: 14903-14908 [PMID: 20668238 DOI: 10.1073/pnas.1008990107]

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