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No evidence for *JAK2* V617F mutation in colorectal cancer

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Colorectal cancer (CRC) is the third most common cancer and the fourth most common cancer cause of death globally,[1] and 40–50% of newly diagnosed patients will develop metastatic disease.[2] Despite therapeutic advances, the prognosis for patients with metastatic CRC remains poor, with a median overall survival of 18–21 months.[3]

The Janus family of tyrosine kinase 2 (JAK2) and the signal transducers and activators of transcription (STAT) family of transcription factors are crucial components of diverse signal transduction pathways that are actively involved in cellular survival, proliferation, differentiation and apoptosis.[4] JAK2 is constitutively associated with many cytokines and is responsible for signalling from various growth factor receptors, and this mutation results in deregulated intracellular signalling with cell proliferation that is independent of normal growth factor control in different types of malignancy.[5]

A somatic point mutation (V617) has been described in the conserved autoinhibitory pseudokinase domain of JAK2 protein, which plays an important role in haematopoietic signalling and in myeloproliferative disorders such as clonal polycythaemia vera, essential thrombocythopenia[6] and chronic idiopathic myelofibrosis.[7] In this V617 mutation, the G→T exchange at nucleotide 1849 in exon 12 of the *JAK2* gene leads to a substitution of valine to phenylalanine at amino acid position 617 of the JAK2 protein within the JH2 pseudokinase domain.[8] This conformational change promotes the constitutive (trans)-phosphorylation of activation loop Y1007 and constitutive kinase activity, although the mutant JAK2 retains its ability to bind the cytosolic domains of cytokine receptors[9] and requires binding dimeric cytokine receptors for full activation. *JAK2* V617F activates multiple signalling pathways that function downstream of the wild-type *JAK2*, such as the STAT3, STAT5, RAS/MAPK and PI3K-Akt pathways.[10]

The Ras/Raf/MAPK and PI3K/Akt pathways are immediately downstream of epidermal growth factor receptors (EGFR) and have been analysed in solid tumours, especially in CRC, as they converge to drive proliferation, survival, angiogenesis, metastasis and invasion. It is also well known that metastatic CRCs respond differently to EGFR-targeted agents and that the tumour-specific response has a genetic basis.

As a consequence, a great deal of recent works has defined mutations in genes included in this pathways as *KRAS* (human homolog of the Kirsten rat sarcoma-2 virus oncogene), *BRAF* (V-raf murine sarcoma viral oncogene homolog B1) and phosphatidylinositol 3-kinase (PI3K) events distinguish malignant from normal cells and make tumours resistant to treatment with anti-EGFR monoclonal antibodies such as cetuximab or panitumumab.[11,12] Despite extensive study, there are patients with wild-type *KRAS, BRAF* and PI3K who do not respond to these treatments. In contrast, *JAK2* mutations have not been studied in solid tumours, including CRC.

Corvinus *et al*.[13] showed that persistent STAT3 activation in CRC is associated with enhanced cell proliferation and tumour growth, and the blockade of STAT3 activation in CRC-derived xenograft tumours slowed their development. In addition, studies in cell lines showed that JAK2 interacted functionally with Raf-1, a central component of the RAS/MAPK pathway. Therefore, it is possible that the *JAK2* V617F mutation may lead to disruption of one or more of these pathways, influencing normal cellular responses and resulting in different disease states in CRC. The role of JAK-STAT mutations in cancer is being explored and it is possible that mutations in components of this pathway are present in various cancers, including CRC.[14]

The identification of this acquired mutation establishes the presence or absence of a clonal disorder and would allow better or new approaches to the diagnosis and treatment of this type of tumour.

Table 1. Patient demographics and baseline tumour characteristics.

Gender	Male	70.8%
	Female	29.2%
Age	Median (range)	68 years (31–88)
Duke's stage	A	10.7%
	B	12%
	C	70.6%
	D	6.7%
Histological Grade	Well differentiated	4.6%
	Moderately differentiated	81.6%
	Poorly differentiated	13.8%

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To date, no survey of the *JAK2* V617F mutation has been undertaken in CRC. This study analyses the *JAK2* V617F mutation in CRC patients using a Taqman allelic discrimination assay, and aims to elucidate whether or not the *JAK2* V617F mutation plays a role in CRC carcinogenesis and is responsible for the absence of response to treatment using anti-EGFR monoclonal antibodies.

This retrospective study included 75 patients with primary colorectal cancer treated at General Yagüe Hospital, Burgos, Spain (age range: 31–86 years [mean: 68]). Clinical data were obtained from the tumour registry and from hospital charts. These data included patient age at diagnosis, gender, Duke's stage and histological grade (Table 1).

For the Taqman allelic discrimination assay, paraffin wax-embedded tumour sections were deparaffinised and air dried, and then DNA was isolated using proteinase K and the QIAamp DNA FFPE tissue kit (Qiagen) according to the manufacturer's protocol. Mutant *KRAS* in exon 2 was detected using a validated *KRAS* mutation kit (DxS, Manchester, UK) following the manufacturer's instructions. This analysis identifies seven somatic mutations located in codons 12 and 13 (Gly12Asp, Gly12Ala, Gly12Val, Gly12Ser, Gly12Arg, Gly12Cys, and Gly13Asp) using an allele-specific real-time polymerase chain reaction (RT-PCR) methods.[15–17] The analysis was performed in an ABI Prism 7500 instrument (Applied Biosystems).

BRAF mutation in exon 15 and codon 600 (V600E) was evaluated using a modification of a method previously reported by Benlloch and colleagues.[18] The primers were designed to avoid amplification of a pseudogene located on the X chromosome. Briefly, a set of primers and probes was used as follow: BRaf-F (forward)

5′CTACTGTTTTCCTTTACTTACTACACCTCAGA-3′: BRaf-R (reverse) 5′ATCCAGACAACTGTTCAAACTGATG-3′, wild-type probe 5′VICCTAGCTACAGtGAAATC-3′ and mutant probe 5′-FAM-TAGCTACAGaGAAATC-3′. The primers and probes were tested with controls of DNA from the HT29 cell line (ATCC), which harbours the V600E *BRAF* heterozygotic mutation, and DNA from the SW480 cell line (ATCC), which is a *BRAF* wild-type *KRAS* mutant.

An RT-PCR method was performed in a final reaction volume of 20 µL containing 10 µL 2x Genotyping PCR Master Mix (Applied Biosystems), 900 nmol/L each primer, 250 nmol/L each probe and 5 µL DNA solution. MicroAmp optical 96-well plates with optical adhesive covers (Applied Biosystems) were used. Amplification and detection were performed using an ABI prism 7500 sequence detection system (Applied Biosystems). The amplification conditions were: 2 min at 50˚C for AmpErase uracil-*N*-glycosylase activity and 10 min at 95˚C for AmpliTaq Gold activation, followed by 50 cycles of 15 sec at 92˚C for denaturation and 1.5 min at 60˚C for annealing and extension. The fluorescence data were analysed using the allelic discrimination software on the ABI Prism 7500 instrument.

A validated kit for *in vitro* diagnostic use (JAK2 MutaScreen Ipsogen) was used that works with a PCR allelic discrimination assay with two Taqman probes in a mulpliplexed assay. The probes were labelled with FAM (mutant allele and VIC [wild-type]) dye. The kit provides positive and negative controls and a cut-off sample. The amplification conditions used were: 2 min at 50˚C and 10 min at 95˚C followed by 50 cycles of 15 sec at 92°C for denaturation and 1 min at 60˚C for annealing and extension. The post-read step was 1 min at 60˚C. The fluorescence data were analysed using the allelic discrimination software on the ABI Prism 7500 instrument (Applied Biosystems).

This study included eight (10.7%) Duke's stage A tumours, nine (12%) stage B tumours, 53 (70.6%) stage C tumours and five (6.7%) stage D tumours. Also included among these cases were some rare subtypes of carcinoma (four mucinous, two signet ring type). Results showed absence of the *JAK2* V617F mutation in the different CRC subtypes using MutaScreen Ipsogen.

In the Spanish population, the *JAK2* V617F mutation does not seem to have a role in CRC tumour development. This retrospective study, which appears to be the first to analyse *JAK2* V617F mutation in wild-type *KRAS* and *BRAF* patients, has shown that this specific mutation is not responsible for the absence of response to anti-EGFR therapy in CRC patients. However, the absence of the *JAK2* V617F mutation does not exclude the presence of other *JAK2* mutations.

The specific missense somatic mutation V617F has been reported in chronic myeloproliferative disorders[19] such as polycythaemia vera, essential thrombocythopenia and chronic idiopathic myelofibrosis.[20]

Although the *JAK2* V617F mutation has not been observed in CRC, mutations in other regulatory molecules within the JAK2 signalling pathway may result in deregulated JAK2 signalling. Constitutive activation of the JAK-STAT pathway can also be induced by mutations in the negative regulators of JAK-STAT signalling, such as the SOCS protein. The involvement of SOCS in the pathogenesis of various cancers has been established recently. Repression of *SOCS1* gene expression has been described for solid tumours in the colon, liver, breast and pancreas.[21–24]

Several studies have suggested that methylation of CpG sequences in the promoter of the *SOCS3* gene leads to silencing of its expression. As *SOCS3* is a strong negative regulator of the JAK-STAT pathway, its absence might enable persistent anti-apoptotic signalling by weak JAK-STAT activation. This might contribute to the survival and progression of several tumours, including hepatocellular carcinoma.[5]

Previously, Kocher *et al*.[25] postulated that the *JAK2* V617F mutation played a role in pancreatic cancer; however, absence of the *JAK2* V617F mutation in CRC tumours was demonstrated in this study.

In summary, this study shows that the *JAK2* V617F mutation is not responsible for CRC development, nor does it affect the efficacy of anti-EGFR therapy in wild-type *KRAS* and *BRAF* patients. These results also suggest that JAK2 is not a promising target for adjuvant therapy in CRC patients. □

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Maternal human papillomavirus (HPV) infection and its possible relationship with neonatal prematurity

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Although human papilloma virus (HPV) infection is considered a sexually transmitted infection, it can also be transmitted by non-sexual routes including perinatal transmission, autoinoculation and heteroinoculation and, possibly, indirect transmission via fomites.[1–3] Acquisition of maternal genital HPV infection by infants at birth has been proposed by several researchers.[2,4,5] Newborn babies are exposed to maternal cervical HPV infection which persists for at least six months.[5] Neonatal infections are predominantly of HPV types 16 and 18 and persist in the neonatal genital area as well as in their oral cavity. In a study by Cason *et al*.[5] the transmission rate of infection from HPV-positive mothers to their infants 24 hours after delivery was approximately 73%. The concordance of different HPV types detected in newborn babies and their mothers also indicates perinatal mother-to-infant HPV transmission.[2]

To date, no published data are available concerning the possible relationship between maternal genital HPV infection and their children's antenatal, perinatal or post-natal history. It remains unclear how frequently perinatal HPV infection progresses to clinical lesions, whether they be genital, laryngeal or oral.[2] Moreover, there appear to be no data regarding the possible relationship between genital maternal HPV infection and neonatal prematurity. Factors that can cause neonatal prematurity include maternal chronic infection (group B streptococcus, *Listeria monocytogenes, Ureoplasma urealyticum, Mycoplasma hominis, Trichomonas vaginalis, Gardnerella vaginalis, Bacteroides* spp.).[6]

Over a four-year period (2002–2006), cervical samples were obtained from 276 mothers after the birth of their first child and tested for the presence of HPV using the polymerase chain reaction (PCR) technique. Women with multiple births were not enrolled in the study. The sample included 146 mothers with a normal Papanicolaou smear, 78 mothers with low-grade squamous intraepithelial lesions (SILs), 45 mothers with high-grade SILs and seven with cervical cancer. Genomic and viral DNA was extracted from all collected tissues or smears and stored at –20°C.

Virus detection was performed using a PCR technique as described previously.[7] Briefly, the extracted DNA (1 μL) of each cervical sample was amplified in a total volume of 30 μL

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