Epigenetics

CIMP and colon cancer gets more complicated

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Evidence for a subset of colon cancers with low-level CIMP that has unique molecular and clinical features compared with cancers with no CIMP and high-level CIMP

Colorectal cancer (CRC) is one of the most commonly occurring cancers in adults, and arises through the cumulative effects of inherited genetic susceptibilities and environmental exposures. These two sets of factors interact to cause CRC by either inducing or permitting the progressive accumulation of gene mutations (such as those in *APC*, the ''gatekeeper'' tumour suppressor gene) and alterations to the epigenome (such as aberrant methylation of *MGMT* or *CDKN2A*). The importance of the accumulation of multiple gene mutations in causing colon cancer is highlighted by the fact that colon cancer can be divided at the molecular level into at least two distinct molecular categories based on the types of mutations observed. These two categories are the chromosome instability (CIN) group, which is characterised by the presence of aneuploidy, chromosome translocations, and chromosomal gains and losses, and the microsatellite instability (MSI) group, which is characterised by the presence of frameshift mutations in repetitive elements of DNA called microsatellite repeats. These molecular subgroups of tumours have different mutation frequencies for certain genes such as *TP53* and *BRAF*, and have unique clinical features, such as the tendency of MSI tumours to occur in the right side of the colon and to have less aggressive clinical behaviour than CIN tumours.[1]

Recently, considerable attention has also been focused on the role of epigenetic alterations of candidate tumour suppressor genes in the molecular pathogenesis of CRC. It is well known that the expression of genes can be affected by gene-promoter methylation and the chromatin structure of the gene locus, which are epigenetic factors that regulate gene expression. In particular, the aberrant methylation of CpG island DNA in gene promoters is a common phenomenon in many cancer types, including gastrointestinal cancer, and silences the expression of tumour suppressor genes. Virtually all colon cancers display at least a low level of aberrant DNA methylation, and a subset of approximately 15–20% of colon cancers show a high level of aberrantly methylated genes.[2]

The observation that a subset of colon cancers appear to commonly methylate genes led Jean-Pierre Issa's research group to propose that there is a distinct molecular subgroup of CRC that has a hypermethylator phenotype.[3] This distinct trait of excessive gene methylation has been termed the CpG island methylator phenotype (CIMP) and is believed to be a distinct molecular subgroup of CRC that is fundamentally different from other colon cancers. It is noteworthy that the existence of CIMP has been a point of substantial controversy in this field, as it has been unclear whether CIMP simply reflects the far end of a continuous distribution of tumours with methylated genes or if it is a unique subgroup of CRC with a distinct molecular aetiology.[4 5] It has been suggested that the identification of a group of tumours that are heavily methylated is a consequence of biased selection of methylated genes and limitations of data-analysis techniques.[5] Indeed, the missing piece of information needed to resolve this argument, the cause of CIMP, still eludes us. Nonetheless, in 2006, Weisenberger *et al* made a substantial contribution to the field by identifying a set of aberrantly methylated genes that clearly define a subgroup of CRCs with an excessive amount of methylated genes.[2] By analysing 195 loci using MethyLight quantitative methylation-specific PCR assays on training and test sets of CRCs, they determined that a five-gene set consisting of *CACNA1G, IGF2, NEUROG1, RUNX3* and *SOCS1* could reliably identify a subgroup of CRCs that have features previously reported to associate with excessive DNA methylation (such as *BRAF* and *KRAS* mutations, MSI, proximal colon location and female preponderance) The identification of this strongly performing panel will hopefully reduce some of the confusion in the field that has resulted from varied criteria being used to characterise tumours as having a CIMP phenotype. Ultimately, selecting a consistent panel of methylated genes that designate a group of CRCs that are ''hypermethylators'' should help investigators determine the root cause of CIMP by allowing studies to be more easily compared. This is essential, as there are a variety of candidate mechanisms that may be the cause of CIMP, such as a fundamental defect in processes that regulate the fidelity of DNA methylation, an underlying genetic defect that causes excess DNA methylation, or a susceptibility to ''epimutagens'', proposed environmental factors that can alter the epigenetic status of genes.[6 7] An additional possibility is that CIMP tumours arise from a different cell of origin in the colonic epithelium from that of non-CIMP CRCs and that the epigenetic alterations observed in CIMP cancers reflect this alternative cancer stem cell.[8 9]

Concurrent with efforts to identify a panel of genes that can consistently identify CIMP tumours, several groups have been correlating molecular and clinical features of CRCs with the hypermethylator phenotype. The study by Ogino *et al*[10] in this issue of *Gut* ***(see page 1564)*** is one of a series of studies of CIMP CRCs that this research group has recently published, which characterise the molecular features of CIMP tumours. This group of investigators has been actively engaged in further defining CIMP tumours at the molecular level though an exhaustive evaluation of CRCs that have been collected through the Nurses' Health Study (n = 121 700 women followed since 1976) and the Health Professional Follow-up Study (n = 51 500 men followed since 1986).[11 12] They used MethyLight assays to assess the methylation status of the CIMP panel and of *CDKN2A* and *CRABP1*, and correlated these results with p27, COX-2, and p53 expression, and with the mutation status of the transforming growth factor-β receptor type II gene (*TGFBR2*).[13–19] Using criteria of ⩾4/5 loci being methylated to define CIMP, they found decreased nuclear p27 (CDKN1B/KIP1) expression in these tumours, especially in those cancers with absent p53 expression, as well as reduced COX-2 expression and an increased frequency of *TGFBR2* mutations. The size of the collection of CRCs that this group has analysed has given them the power to stratify the tumours on multiple molecular endpoints and to sort the CRCs into discrete subgroups based on these molecular features. These findings provide more support that there is a CIMP category of

CRC that is unique at the level of its molecular pathogenesis. However, the findings still do not provide any additional insight into the underlying cause of CIMP.

Now, to add to this evolving knowledge base on CIMP tumours, Ogino *et al* have begun to make the case that there is a group of tumours that shows an intermediate amount of aberrant DNA methylation, which they have termed ''CIMP-low''. Based on a panel of markers that include eight genes (*CACNA1G, CDKN2A, CRABP1, IGF2, MLH1, NEUROG1, RUNX3*, and *SOCS1*), they have defined CIMP-low tumours as tumours with >1/8 and <5/8 methylated genes as measured by a panel of MethyLight assays. They have found that tumours that are CIMP-low and that have low levels of MSI frequently carry methylated *MGMT*. Moreover, they have found an inverse relationship in CIMP-low tumours between MSI-high (>40% of loci showing MSI in the NCI Bethesda consensus panel) and methylated *MGMT*. These results are consistent with earlier results from this group that found a direct correlation between CIMP-low tumours and male sex and *KRAS* mutations, and support the idea that CIMP-low tumours are another discrete subgroup of CRCs (table 1).[20]

What are the implications of the results of this study and those of studies of CIMP in general for our understanding of the pathogenesis of CRC? There are two main implications of CIMP and CIMP-low for our current understanding of the molecular biology of CRC. The first is that the finding that CIMP is probably a true molecular subgroup of CRC supports another evolving concept regarding colon cancer, which suggests that some CRCs originate from hyperplastic polyps rather than from adenomas. Until recently, it was believed that only conventional tubular and tubulovillous adenomatous polyps had the potential to undergo malignant transformation; however, it now also appears that a subset of CRCs can evolve from hyperplastic polyps.[21] These hyperplastic polyps that have the potential to undergo malignant transformation appear to do so by evolving into serrated polyps, which can then transform into cancer. Thus, a subset of hyperplastic polyps appears to have the potential to transform into adenocarcinomas through a hyperplastic polyp→serrated adenoma→adenocarcinoma progression sequence.[21–23] It is suggested that CRCs arising through a hyperplastic polyp→serrated adenoma→CRC pathway have a unique molecular and histological pathway through which they arise.[23] Serrated polyps commonly display CIMP and *V600E BRAF* mutations, which are correlated with CIMP. These findings suggest that CIMP CRCs arise from serrated polyps, which in turn may arise from a stem-like cell that is different from the stem-like cell of origin that gives rise to CRCs developing from tubular adenomas. In fact, Jass has termed the hyperplastic polyp→serrated adenoma→CRC pathway a methylator pathway.[23] If further studies can confirm that there is a biologically unique category of CRCs that display CIMP-low, it will need to be determined whether these tumours arise from adenomas or hyperplastic polyps, or if they follow a third, currently unrecognised pathway to colon cancer. Clearly, this is an exciting line of investigation, but additional studies are needed to validate these concepts.

The second major implication of the studies of CIMP tumours and the suggestion of a CIMP-low as well as a CIMP-high category of tumours is related to the fundamental cause of aberrant DNA methylation in cancer. The current most strongly supported models of the underlying mechanism of CIMP are that aberrant CpG island methylation occurs as the result of an underlying genetic defect or that it arises from the effect of epimutagens. Possible genetic causes include activating mutations in DNA methyltransferases, (although there is no support for this to date) or alterations in genes that control mechanisms that protect DNA from aberrant methylation. Turker *et al* have demonstrated that there may be ''methylation centres'', which are sequences that attract DNA methyltransferases, from which cancer-related aberrant DNA methylation can spread into regions whose protecting ''boundary elements'' have been breached. This model argues that the methylation occurs as the consequence of deregulation of local factors in *cis*-DNA (eg, methylation control centres, such as SP1 sites or tandem B1 elements) that culminate in the aberrant methylation of tumour suppressor genes. However, a second model is that there are environmental exposures, termed epimutagens, that can cause aberrant DNA methylation.[24 25] Indeed, Kikuchi *et al* have demonstrated that exposure to tobacco smoke is significantly associated with methylation of *CDKN2A/p16* in non-small cell lung cancer, reinforcing the role of environmental agents in mediating this class of epigenetic alterations.[26 27] It is also likely that the genetic and epigenetic alterations may cooperate to promote tumour formation and that detection of colon adenomas with methylation may identify colonic epithelium that is at significant risk of acquiring genetic alterations that will lead to colon tumour formation (ie, a field defect related to exposure to epimutagens that prime the tissue for cancer formation).[28 29]

In summary, CpG island methylation is of particular interest in cancer formation, not only because it is an alternative mechanism to gene mutation for silencing tumour suppressor genes, but also because there appears to be a unique subgroup of CRCs that display excessive DNA methylation and have molecular and clinical traits that distinguish them from other CRCs. They may also arise from a unique underlying cause that occurs as part of a field cancerisation process that predisposes tissue to neoplastic transformation.[30–33] The concept of CIMP-low and CIMP-high colon cancers would fit well with an epimutagen model of CIMP cancer in which the degree of CIMP is a reflection of the exposure level to the epimutagens. The studies by Ogino *et al* provide more information to help better understanding of CIMP and hopefully, will inform studies that ultimately will identify the mechanism responsible for aberrant DNA methylation in cancer. However, for now they introduce additional complexity into an area of cancer biology that seems to have more questions than answers.

Table 1 Features of CRCs based on CIMP status

Features	Non-CIMP	CIMP-low	CIMP-high
Tumour location	Distal > proximal	—	Proximal > distal
Gender bias	Male = female	Male>female	Male < female
BRAF mutation status	Wild type	Wild type	Mutant
KRAS mutation status	Wild type	Mutant	Wild type
Genomic instability status	CIN	Similar to non-CIMP	MSI is common

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Liver transplantation

Intercontinental comparison of patient cohorts: what can we learn from it?

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Survival after liver transplantation in the United Kingdom and Ireland compared with the United States

Dawwas *et al* have published an impressive paper (***see page 1606***),[1] which compares 90-day mortality, mortality between 90 days and the first year, and long-term survival beyond the first year in patients after primary liver transplantation (LTx) performed in the United States (USA) with that performed in the United Kingdom (UK) and Ireland between 1994 and 2005. For their analysis they used the corresponding transplant databases, such as the Liver Transplant Audit and the Organ Procurement and Transplant Network/United Network for Organ Sharing (OPTN/UNOS), respectively. After careful modification, both databases were harmonised in order to perform an adequate statistical analysis.

The main finding of their analysis was that the 90-day mortality was significantly higher in UK/Ireland than in the USA, both in patients receiving a transplant for acute liver failure and in patients with chronic liver disease. In contrast, patients who survived the first years after LTx in the UK/Ireland had a lower overall risk-adjusted mortality than their counterparts in the USA. Based on these findings, the authors have concluded that the USA has better acute perioperative care than the UK/Ireland, whereas UK and Ireland seem to provide better quality long-term care after LTx.

Overall, the study itself was well planned and designed. The authors made sure that the data were harmonised as necessary, and only patients with a defined number of complete datasets were included. It is known that with such large databases there is a problem with data quality and transfer, but the overall quality of both transplantation databases is generally accepted. Thus the databases should not be considered responsible for the described differences in survival. If, therefore, the data quality and its analysis are considered to be adequate, then other factors need to be reviewed to explain the differences, especially differences in short-term survival.

The authors discussed some of these factors but failed to provide answers to the key question: how can transplant teams in the UK/Ireland adapt their treatment regimens in order to improve short-term survival in patients undergoing LTx? Thus, based on the article by Dawwas *et al*, this commentary discusses key concerns in liver transplantation, such as medical expertise, patient cohorts, organ/graft quality and organ availability for the two different patient cohorts—that is, USA and UK/Ireland. There is no doubt that, in general, survival of patients after LTx both in the USA and UK/Ireland has greatly improved over the years.[2–4] It is widely accepted that this improvement is based