Colorectal Cancer: Molecular Classification And Clinical Application

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Abstract— Genetic -depth study showed the perplexity of molecular heterogeneity of colorectal cancer (CRC). Though various therapies exist, we do not have the proper way to choose the right treatment for each patient, personalized treatment strategies are in demand. For CRC, a broad molecular classification is still missing. We wish to apply the molecular techniques to improve the outcome. Our intention in this review is to summarize the molecular classification of CRC and their reflection on management.

Index Terms— Colorectal cancer, molecular classification, colon cancer subtypes.

I. INTRODUCTION

Colorectal cancer (CRC) is one of the major causes of morbidity and mortality, more than 1.2 million patients are diagnosed every year, and more than 600,000 die from the disease, more common in old age men; median age at diagnosis is about 70 years in developed countries [1]. CRC is not one disease, although with the same stage of CRC, the response to treatment may be different; could be explained by the molecular heterogeneity, either genetic or epigenetic. In spite of great interest, the molecular classification of CRC has not achieved widespread clinical application, and has not been approved by many oncology centers. Better understanding of molecular classification will help us to assimilate the process of carcinogenesis and may contribute to create novel and more effective therapy [2]. In this review, we summarize the molecular pathways and classification of CRC and the impaction on patients.

II. EVOLUTION OF MOLECULAR CLASSIFICATION OF CRC REVIEW STAGE

The transformation from normal epithelium to carcinoma is associated with many molecular events. In CRC carcinogenesis, there are three major distinguished molecular pathways have been involved: chromosomal instability (CIN), microsatellite instability (MSI), and CpG island methylator phenotype (CIMP) [3].

The first and most common (70%), the CIN pathway, is characterized by frequent loss of heterozygosity (LOH) in chromosomes, alternation in the main oncogenes (e.g., KRAS, NRAS, BRAF, PIK3) and tumor suppressor genes (e.g., APC, TP53, and PTEN). Key pathways include Wnt/β-catenin, transforming growth factor beta (TGF-β), epidermal growth factor receptor (EGFR, HER1), downstream mitogen-activated protein kinase (MAPK), and phosphoinositide 3-kinase (PI3K) signaling activation [4].

The second pathway is the MSI, occurs in 15% of CRC and caused by inactivation of DNA mismatch repair genes (MMR). The presence of MSI represents ontotypic evidence that MMR is not functioning normally (d MMR). CRC with MSI has a clear molecular origin and a specific clinicopathological phenotype; associated with poor differentiated tissue, high mucinogens, tumor infiltrating lymphocytes, right-sided location, and enriched with BRAF mutations [5].

In addition to CIN and MSI, a third epigenetic instability pathway (found in approximately 15%–20% of CRC) was explained by Toyota et al. CpG island methylation phenotype (CIMP), characterized by vast hypermethylation of promoter CpG island sites, resulting in the inactivation of several tumor suppressor genes or other tumor-related genes [6]. The accurate description of CIMP has not been equal among studies; actually there are different classifications for CIMP tumors. Table 1 illustrates some of these classifications.

The three molecular pathways are not mutually exclusive, so they may be exhibited in the same CRC patient. Actually, there are many proposals for molecular classification of CRC but without complete agreement. According to clinical, morphological, and molecular parameters, Jeremy Jass proposed a model included five subgroups [7]. The Cancer Genome Atlas (TCGA) subcategorized CRC depending on mutation rate into hypermutated and nonhypermutated group [8].

Another classification system was published by Domingo et al, dividing CRC into 7 groups [9]. Based on genetic and clinicopathological characteristics, De Sousa E et al. identified 3 different subtypes of CRC [10]. In other study done by Sadanandam et al, 5 different types of CRC were identified based on gene expression profiles [11]. Noticeable thing that, BRAF mutations were found in sessile serrated adenoma (SSA) either in early hyperplastic polyps or in the advanced dysplastic form, reflecting its role in neoplastic progression [12].

Importantly, over the last decade, it has been documented that other pathways are implicated in the pathogenesis of CRC, as microRNA (miRNA) and inflammatory pathways. The early reports about miRNA denoting its low expression...
level in cancer, anticipated that they were tumor suppressors. In CRC it is not the truth, more microRNAs have been elevated [13].

Table (2) illustrates some of molecular classifications CRC.

Trezic et al, has established the relation between inflammation and CRC either sporadic or heritable types. Recently, the role of immune mediators has been clarified in CRC carcinogenesis, from tumor initiation till metastasis. The proposed mechanisms may include production of many angiogenic factors hand in hand with DNA damage [14].

III. CLINICAL APPLICATION

The rapid evolution in the identification of molecular basis of CRC has led to discovery of novel drugs and molecular diagnostics markers. The clinical use of monoclonal antibodies (mAbs); cetuximab/ panitumumab, targeting EGFR is an excellent example; moreover, nearly all patients whom initially respond inevitably become refractory [15].

The mechanisms of resistance to EGFR mAbs in CRCs include, range from genetic alterations in the pathway to amplification of receptor tyrosine kinases (TKR). The mechanisms may be assigned as primary resistance as genetic alterations in the RAS-RAF-MEK pathway, HER2 amplification, and MET amplification or acquired resistance such as the EGFR mutation S492R [16]. Moreover, Cetuximab have antibody-dependent cell-mediated cytotoxicity (ADCC) which depends on the interaction between antibody Fc portion and Fc receptors (FcyRs) in immune cells. Bibeau et al, [17] demonstrated that combined FcRIIA and FcyRIIIa polymorphisms are prognostic factors for progression-free survival in mCRC patients treated with cetuximab plus irinotecan, which is corresponding to the study done by Kjersem et al, [18].

Whereas polymorphisms are clinically linked to mutated-KRAS mCRC, an important role of ADCC in cetuximab efficacy is assumed. However, due to retrospective studies most always are criticized as the completeness of data often is suboptimal and depends totally on medical documentation, ancillary studies to larger prospective clinical trials are needed to assess the impact of Fcy R polymorphisms on cetuximab efficiency.

Also, pioneering work demonstrated that antibodies containing engineered bisected increase ADCC amplification. This therapeutic mechanism is likely to be important when simple interference with receptor/ligand interactions fails as a therapeutic strategy [19], and this strategy is undergoing clinical validation.

Diaz et al, detected the KRAS mutations not only in tumor biopsies but also in circulating tumor DNA (ctDNA) in patients with acquired resistance [20]. This may change the method of genetic alteration detection from multiple tumor biopsies to just drawing a tube of blood, what is called liquid biopsies or ctDNA, which is a specific cancer biomarker that can be detected, measured, and tracked. Preliminary data suggests ctDNA is detectable at diagnosis in the majority of patients with non-metastatic CRC. The potential for ctDNA as a CRC screening tool, and as a prognostic marker for early stage, should be further explored [21].

Surprising, not all mutations in KRAS gene have the same biologic behavior. In an analysis, use of cetuximab was associated with longer overall and progression-free survival among patients with chemotherapy-refractory CRC with p.G13D-mutated tumors than with other KRAS-mutated tumors [22, 23].

Considerable preclinical data have shown that the combination of ERBB tyrosine kinase inhibitors and anti-EGFR mAbs leads to markedly higher antitumor activity than the administration of single agents, especially in KRAS wild-type and Quadruple-negative (KRAS/NRAS/ERBB/PIK3CA wild-type) tumors [24- 26].

There are a lot of studies accused HER3 signaling pathways activation and compensatory PI3K pathway activation as a cause of anti-EGFR therapeutics failure. Preclinical cancer models have indicated that patritumab (fully human anti-HER3 monoclonal antibody) demonstrates antitumor activity when used alone or with anti-EGFR inhibitors by binding to the extracellular domain of HER3 and promoting the internalization and degradation of the receptor [27].

With limited clinical benefit from the use of BRAF inhibitors as single agents in BRAF V600E-mutated CRC, the clinical trials tend to investigate BRAF inhibitors in combinations; either with anti-EGFR mAbs or with third agent (MEK or PI3K pathway inhibitors) [28, 29].

Programmed death 1(PD1) and its ligand (PD-L1), are highly expressed in a variety of cancers and hence the role in cancer immune therapy is well established [30]. Gatalica, et al, presented a poster in 2014 ASCO Annual Meeting, concluded, the expression of PD-1 and PD-L1+ cancer cells are more frequent in MSI-H than in MSS CRC, which are rare in general CRC population, subsequently the use of anti-PD-1 mAb perhaps hold new hope for treatment [31]. We summarized some clinical trials in CRC in Table 3.

In a retrospective multicenter study including 782 patients with CRC, post surgery, revealed that the combination of PIK3CA mutations with MSS were associated with good prognosis and postulated that they may not require adjuvant chemotherapy [32].

Reimers et al, observed that, the benefits of the adjuvant aspirin were belonged to CRC patients with COX-2–positive or PIK3CA mutation–negative [33].

MSI is an important piece of information to consider when deciding adjuvant chemotherapy in stage II CRC. Data from the PETACC3 trial suggested that MSI-H tumors have a decreased like hood of metastasis, which is considered as prognostic marker for favorable outcome [34-36]. A retrospective study involving long term follow up of patients with stage II and III CRC have found that patients with stage II MSI-H tumors not only did not derive any benefit from 5-FU adjuvant therapy, but they actually fared worse if they were treated [35]. Similar results were showed by Sargent et al, [36]. In contrast to these finding, a study done by Hutchins et al, from QUASAR study showed that although MSI-H was a prognostic, it did not predict benefit from or detrimental effect on chemotherapy [37]. This corresponding to study done by Bertagnolli et al, on patients in the CALGB and 89803 trials [38].
Although the overall result of National Surgical Adjuvant Breast and Bowel Project protocol C-08 was negative, the data suggest that there may be a subset of CRC patients may get clinical benefit from the addition of bevacizumab to adjuvant chemotherapy, but it needs independent validation in other clinical trials [39].

MicroRNAs are endogenous posttranscriptional modulators that control the expression of the target genes and play an important role in the development and progression of many malignancies, including CRC. Toiyama et al, revealed that Serum miR-21 is a promising biomarker for the early detection and prognosis of CRC [40]. Several studies have shown an association between elevated levels of miR21 and the down regulation of tumor suppressor genes, this has led to miR21 being considered a promising therapeutic target for treating CRC [41].

Table 1: CRC classification based on CIMP status

<table>
<thead>
<tr>
<th>Reference</th>
<th>Method of evaluation</th>
<th>Types</th>
<th>Clinical notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wiesenerger et al.</td>
<td>Methy light technology</td>
<td>- CIMP-positive</td>
<td>- A great correlation of CIMP cancers with BRAF mutations.</td>
</tr>
<tr>
<td>(42)</td>
<td></td>
<td>- CIMP-negative</td>
<td></td>
</tr>
<tr>
<td>Shen et al. (43)</td>
<td>Methy light technology</td>
<td>- CIMP-positive (1.2)</td>
<td>- CIMP1 tumors are often MSI tumors (80%), and have BRAF mutations (53%).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- CIMP-negative</td>
<td>- CIMP2 tumors have KRAS mutations (92%), rarely are MSI or have BRAF or TP53 mutations</td>
</tr>
<tr>
<td>Ogino et al. (44)</td>
<td>Quantified DNA methylation in five CIMP-specific gene promoters (CACNA1G, CDKN2A (p16), CRABP1, MLH1, and NEUROG1)</td>
<td>- CIMP-low (CRC with 1/5 to 3/5 methylated promoters)</td>
<td>-CIMP-low CRC is associated with male sex and KRAS mutations.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- CIMP-high (4/5 or 5/5 methylated promoters)</td>
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<td></td>
<td></td>
<td>- CIMP-0 (0/5 methylated promoters)</td>
<td></td>
</tr>
<tr>
<td>Barault et al. (45)</td>
<td>Quantified DNA methylation in five CIMP-specific gene promoters (hMLH1, p16, MINT1, MINT2, and MINT31)</td>
<td>- No CIMP-Low</td>
<td>- Methylation is an independent prognostic factor in MSS CRC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- CIMP-Low</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- CIMP-high</td>
<td></td>
</tr>
<tr>
<td>Ang et al. (46)</td>
<td>GoldenGate® methylation array</td>
<td>- CIMP-Low</td>
<td>- In comparison to CIMP-L, tumors, CIMP-H tumors were more often located in the proximal colon and showed more frequent mutation of KRAS and B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- CIMP-mid</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- CIMP-high</td>
<td></td>
</tr>
</tbody>
</table>

| Yagi et al (47) | Methylated DNA immunoprecipitation-on-chip | - Low-methylation epigenotype |
|                |                                       | - Intermediate methylation epigenotype |
|                |                                       | - High methylation epigenotype |
|                |                                       | - Three methylation epigenotypes exist in colorectal cancer, and suitable classification markers have been developed. Intermediate-methylation epigenotype with KRAS-mutation (+) correlated with worse prognosis. |

Table 2: Some General Molecular CRC Classification

CRC, colorectal cancer; CS, chromosomally stable; CIN, microsatellite instability; MSI, microsatellite instability; MSS, microsatellite stability; CIMP, Cpg island methylator phenotype; CCS, colon cancer subtypes; DFS, disease-free survival; SSA, sessile serrated adenoma; TA, transit-amplifying; CR-TA, cetuximab-resistant TA; CS-TA, cetuximab-sensitive TA; DFS, disease-free survival;
Table 3: Selected Clinical Trials In Advanced CRC Based On Biological Hallmarks.

<table>
<thead>
<tr>
<th>NCT ID</th>
<th>Total description</th>
<th>Study type and design</th>
<th>Total phase</th>
<th>Last updated</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT0223867</td>
<td>To evaluate the efficacy of DHAMM in patients with advanced colorectal cancer.</td>
<td>International</td>
<td>Phase II</td>
<td>April 30, 2015</td>
</tr>
<tr>
<td>NCT02955204</td>
<td>To determine the molecular behavior of KRAS G12D as an independent marker of disease progression in patients with metastatic colorectal cancer.</td>
<td>International</td>
<td>Phase II</td>
<td>May 4, 2015</td>
</tr>
<tr>
<td>NCT03379357</td>
<td>To evaluate the efficacy of DHAMM in patients with advanced colorectal cancer.</td>
<td>International</td>
<td>Phase II</td>
<td>March 31, 2017</td>
</tr>
<tr>
<td>NCT01897955</td>
<td>To evaluate the efficacy of DHAMM in patients with advanced colorectal cancer.</td>
<td>International</td>
<td>Phase II</td>
<td>March 27, 2019</td>
</tr>
<tr>
<td>NCT01897955</td>
<td>To evaluate the efficacy of DHAMM in patients with advanced colorectal cancer.</td>
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**CONFLICT OF INTEREST**

The authors certify that there is no actual or potential conflict of interest in relation to this article.

**REFERENCES**


