Precision Medicine: Practical Aspects for the Modern Surgeon

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INTRODUCTION

Currently, CRC is not considered a single disease entity, but a heterogeneous group of tumors, both intertumorally and intratumorally. Given the complex molecular composition of these tumors, in recent years efforts have focused on identifying possible molecular markers that provide information on the tumor's response to anticancer therapies, which in turn would help to tailor treatment for CRC. "Predictive" biomarkers identify patients who are most likely to benefit from treatment, and therefore can play a critical role in guiding treatment decisions.

This article will describe the most recent and applied advances in the molecular stratification of CRC with clinical utility.

WHAT IS PRECISION MEDICINE?

Precision medicine aims to personalize medical care with decisions and treatments adapted to each individual. Although genomic analysis is still a relatively new development in drug treatment, this field is expanding rapidly. Currently, more than 200 drugs have information on their labels about pharmacogenomic biomarkers, measurable or identifiable genetic information that can be used to individualize the use of a drug.

In CCR, Precision Medicine focuses on predictive biomarkers. These are biological factors that provide information on the probability of the tumor response to a certain therapeutic agent (unlike the prognostic factor that provides prospective information on the patient's evolution). In CRC these biomarkers are related to the genes involved in colorectal carcinogenesis and its mutations.

The use of these biomarkers is already a standard treatment in metastatic CRC. It is necessary that all doctors involved in the management of these patients have the basic knowledge that allows them to actively participate in decision-making in multidisciplinary teams.

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IMPACT OF TUMOR HETEROGENEITY

CRC results from an accumulation of mutations that give it a selective advantage of local growth in relation to normal cells, and the ability to metastasize. Although there are hundreds of mutations, some of them are determinants for the processes of initiation, progression and metastasis and are known as "drivers" to differentiate them from much more secondary mutations or "passengers" that are not influential in the mentioned processes. Each tumor has 2 to 6 driver mutations in the APC genes (in 80% of tumors), TP53 (50%), KRAS (35%-45%), PIK3CA (20%-30%), and BRAF (10 %).

CRC is a disease that presents great tumor heterogeneity at several different levels. First, the genetic composition of a given tumor type can vary significantly from one patient to another. Also in an individual patient, there is a high degree of genetic heterogeneity between the primary tumor and metastasis, as well as between different metastatic sites (intertumoral heterogeneity). Heterogeneity can exist in different areas within the same tumor (intratumoral heterogeneity), or in different regions of the same patient (spatial tumor heterogeneity). It can also occur at different times in the same tumor (temporal tumor heterogeneity) due to the ability of different clones to resist therapies.

These different levels of tumor heterogeneity can affect the signaling of multiple key oncogenic pathways in a extensive phenotypic variation, such that each tumor, or even within the same tumor, shows its own genetic, epigenetic, transcriptomic and proteomic pattern. Consequently, intratumoral heterogeneity poses a huge challenge to the practice of personalized medicine because a given therapeutic agent targeting a specific molecular agent is likely to impact only a minority of patients.

COLORECTAL CARCINOGENESIS: MICROSATELLITE INSTABILITY AND ITS IMPLICATIONS

There are two types of genomic instability: the most common type is chromosomal instability (present in 80-

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85% of sporadic CRCs), which is a very efficient mechanism in causing alterations in genes such as APC, P53 and SMAD4. The other type is microsatellite instability (MSI), which, although it is characteristic of Lynch syndrome tumors, is present in around 15% of sporadic tumors and has important prognostic and predictive implications for response to treatment.

MSI is considered the molecular fingerprint of a defect in the repair of errors that occur during DNA duplication (DNA mismatch-repair defects). The system that repairs these defects is composed by the repair genes MLH1, MSH2, MSH6 and PMS2. These genes can be affected by inheritable germline mutations (producing Lynch syndrome), and in the exclusive case of MLH1, by non-inheritable methylation that results in their inactivation. In both cases, there is an accumulation of base pairing errors in regions called microsatellites, mainly affecting genes such as TGF-BR2 (transforming growth factor b receptor type II) and BAX (BCL2-associated X protein).

MSI CRCs are more frequently located in the proximal colon, are poorly differentiated, and have mucinous or medullary histology. Its diagnosis is based on the immunohistochemical study of the proteins encoded by the repair genes MLH1, MSH2, MSH6 and PMS2 in the tumor tissue.

These tumors are also considered highly immunogenic given their high mutational load, which results in the formation of tumor-specific neoantigens and T cell infiltration. This explains the association of with a favorable prognosis in patients with early stage colon cancer, and its greater sensitivity to new immunotherapeutic agents such as the anti PD-1 pembrolizumab and nivolumab.

In a phase II study with pembrolizumab the disease control rate was 89% in the MSI tumor group and 16% in tumors without MSI. The CheckMate study of 45 patients verified the first-line efficacy of nivolumab with low-dose ipilimumab in metastatic CRC with MSI-H/dMMR. The objective response rate was 60%, the disease control rate was 84%, and 7% of the patients had a complete response. At 12 months progression-free survival and overall survival were 77% and 83%, respectively. Based on this evidence, and despite the small size of these studies, the FDA has approved pembrolizumab and nivolumab for use in unstable tumors that have progressed after chemotherapy.

BRAF GENE V600E MUTATIONT

The study of the BRAF gene is one of the most frequently used determinations when microsatellite instability is detected (more specifically, expression deficit of the MLH1 repair gene). The V600E mutation represents more than 80% of the alterations, which facilitates the study and reduces costs. When the BRAF is mutated, there is an inactivation (acquired through aberrant methylation) of the MLH1 and the tumor acquires microsatellite instability. The presence of a V600E mutation is mutually exclusive of an MLH1 germline mutation, so its detection practically rules out Lynch syndrome. In addition to this important diagnostic implication, the BRAF V600E mutation is a predictor of the response to panitumumab and cetuximab which is highly unlikely unless BRAF inhibitors are administered (schemes not yet approved for CRC).

K-RAS

The RAS gene family is composed of three genes: KRAS, NRAS and HRAS. KRAS mutations are present in 30%-45% of CRCs (exons 2, 3, and 4). Another 5% of CRCs have activating mutations in NRAS (in the same exons as KRAS). The role of RAS mutations as a negative predictive marker for anti-EGFR therapy in the treatment of mCRC has been proven in multiple clinical trials. Studies have also clearly established that the addition of anti-EGFR therapy (e.g. Cetuximab and Panitumumab) to chemotherapy does not improve results and is even potentially harmful.

FUTURE PERSPECTIVES

New emerging biomarkers

There is a wide variety of biomarkers that are currently being evaluated and do not yet have evidence for clinical application. The PIK3CA mutation (present in 10-18% of CRCs) has evidenced as a negative predictor of responses to anti-EGFR agents. HER2 amplification has a potential predictive value but its prevalence is extremely low and the value of loss of expression of PTEN and CDX2 is controversial. Although it is expected that molecular subtypes may be predictive, no assessment is yet available. Micro RNAs (miRNAs) are short non-coding sequences (18-22 nucleotides) involved in the regulation of gene expression and probably in colorectal tumorigenesis and are also being evaluated as predictive or prognostic factors.

Liquid biopsy

New technologies allow the isolation of different circulating tumor components in the blood (e.g. tumor cells, tumor DNA, exosomes, microRNAs) that can provide useful information for diagnostic, prognostic and predictive purposes. The study of liquid biopsy has acquired a prominent place in cancer research (especially in the lung), and one of the characteristics that makes it especially pro-

mising is its ability to overcome the problem of tumor heterogeneity. Unlike tumor tissue biopsy, which may not be representative of the entire tumor, liquid biopsy can not only be more representative, but also allows following the evolution of the tumor in real time. In a study by Morelli et al., the analysis of plasma collected in 60 patients refractory to anti-EGFR mAbs and without initial KRAS mutation revealed the presence of KRAS mutation in 44% of cases. These new mutations detected in patients refractory to anti-EGFR treatment may derive from rare and pre-existing clones in primary tumors.

The Multiomic Approach

Large-scale "omic" techniques have the ability to identify tumor biology with an extraordinarily high definition that allows understanding of complex pathologies and discriminating tumors with different molecular phenotypes. Transcriptomics is the study of the overall expression of mRNA for a particular tissue. Epigenetics is the study of epigenetic modifications that have an important role in carcinogenesis and are frequent in CRC. These alterations include methylation of the DNA promoter region and modifications in histones. Proteomics studies proteins on a large scale to map biological processes involved in carcinogenesis. Finally, genomics, based on advances in Next Generation Sequencing (NGS) technology that allow the study of millions of DNA bases, is providing a great opportunity for biomarker development and realization of precision medicine in CCR.

Molecular epidemiology

In addition to the heterogeneity that characterizes CRC due to its notable genetic instability, another great barrier to the development of precision medicine is the action of exogenous factors such as lifestyle, diet, nutrition, environment, and microbiome. These factors also influence the pathogenesis and behavior of CRC by acting on not only tumor cells, but also in non-neoplastic cells, including immune cells (microenvironment). Hence the importance of investigating the interactive effects between tumors and all exposures (called exposomes). This has become a new field of research known as molecular epidemiology, the aim of which is to provide new insights into environment, tumor, and host interactions to better understand how endogenous and exogenous factors modify tumor phenotypes.

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