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Nanotechnologies for the treatment of colon cancer: From old drugs to new hope

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ABSTRACT

Colorectal cancer is a wide-reaching health problem due to its incidence and to the high mortality rates. Adjuvant chemotherapies have considerably improved the prognosis and/or the overall survival of patients with locally advanced and metastatic cancers. Nevertheless, their efficiency remains limited due to intrinsic and emerging multidrug resistance (MDR) of cancer cells, and to major adverse effects and dose limiting toxicities. The present review discusses the knowledge of clinically relevant mechanisms of resistance to cytotoxic and targeted therapies for the treatment of colorectal cancer, and focuses on the benefit of nanomedicine approach to circumvent these processes. Nanomedicaments should allow extensive cancer cell drug loading independent on cell surface transporters, –thus overwhelming drug metabolism and efflux-, but also alleviate side-effects related to tissue-dependent drug uptake. Finally, we provide an outline of preclinical and clinical studies of nanoparticles formulations for colorectal cancer treatment, and briefly discuss strategies to optimize the selective delivery of these nanomedicines to colorectal cancer cells.

1. Introduction: incidence of colorectal cancers, molecular characteristics, evolution

Colorectal cancer (CRC) is a major cause of cancer morbidity and mortality. Nearly 135,000 and 450,000 individuals are diagnosed annually in United-States and in Europe, where this cancer is responsible of approximately 50,000 and 215,000 related deaths, respectively (Ait Ouakrim et al., 2015; Siegel et al., 2016). In line with other malignancies, metastases are the main cause of colorectal cancer-related mortality. These distant metastases mainly develop in the liver, where they are evidenced in approximately 25% of patients at the time of diagnosis, whereas metachronous metastatic disease arises for 20–25% of patients, resulting in a relatively high overall mortality rate. When colorectal cancer is localized, the five-year survival rate is about 90% but it falls to nearly 12% once there are distant metastases (Siegel et al., 2014).

The onset of CRC is linked to intrinsic risk factors, such as aging, gender –risk slightly higher for male-, and genetic disposition. About 75% of new cases of CRC –termed sporadic- occur in absence of known predisposing factors, whereas nearly 20% of new cases of CRC arise in a context of familial history of CRC without identified genetic defect. Hereditary non polyposis colorectal cancer (HNPCC) and familial adenomatous polyposis (FAP) are dominant inherited autosomic cancer syndromes that account for 5% and 1% of cases, and are related to the defect in DNA mismatch repair genes and the inactivation of the *APC* gene, respectively (Bronner et al., 1994; Kinzler et al., 1991; Nicolaides et al., 1994; Nishisho et al., 1991; Papadopoulos et al., 1994). Other rare inherited colorectal cancer

Abbreviations: ABC transporter, ATP-binding cassette transporter; CAF, cancer-associated fibroblasts; CSCs, cancer stem cells; CTR1, Copper transporter 1; CIMP, CpG island methylator phenotype; CRC, colorectal cancer; DPD, dihydropyrimidine dehydrogenase; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; EPR, enhanced permability and retension effect; HGF, hepatocyte growth factor; HNPCC, hereditary non polyposis colorectal cancer; mCRC, metastatic colorectal cancer; MMPs, matrix metalloproteinases; MRP2, multidrug resistance protein 2; MSI, microsatellite instability; MSS, microsatellite stable; NPs, nanoparticles; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; OHP, OXA, oxaliplatin; OPRT, orotate phosphoribosyltransferase; OS, overall survival; P-gp, P-glycoprotein; RCT, radio-chemotherapy; SLC, solute carrier; TAM, tumor-associated macrophages; TGF-β, transforming growth factor-beta; 5-FU, 5-Fluorouracyl.

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syndromes characterized with a lower penetrance include the recessive MUTYH-associated polyposis caused by mutation in human Mut Y homologue *MUTYH* –a base excision repair system that protects against transversion G:C/A:T following oxidative DNA damage-, and dominant syndromes, *e.g.* Peutz-Jeghers (*LKB1* inactivation), Juvenile Polyposis syndrome (*BMPR1A* or *SMAD4* inactivation), Cowden syndrome (*PTEN* inactivation 85% cases, decreased activity of KILLIN and SDH B/D, or PIK3CA and AKT1 activation) and mutations in the proofreading domain of DNA polymerase epsilon and delta1.

Some chronic diseases are associated with an increased risk of colon cancer, these include Inflammatory Bowell Disease (mainly ulcerative colitis), obesity and diabetes (Ma et al., 2013; Tsilidis et al., 2015; Ullman and Itzkowitz, 2011). The imbalance in intestinal microbiota and dysbiosis through the induction of inflammation, the release of reactive oxygen species and microbia-derived metabolism or toxins are also suspected to promote genetic and epigenetic alterations leading to CRC (Irrazabal et al., 2014).

On the other hand, environmental and lifestyle factors, such as smoking, alcohol use, and high fat Western diet have been clearly linked to polyp development and CRC (Center et al., 2009; Chen et al., 2015; Cross et al., 2010; Bardou et al., 2002; Botteri et al., 2008a,b; Huxley et al., 2009).

2. Molecular mechanisms involved in colorectal carcinogenesis

The evolution and heterogeneity of cancer cells might be depicted by two models. The clonal evolution model hypothesizes that all cancer cells are tumorigenic and accumulate stochastic genetic/epigenetic alterations allowing the expansion of a subpopulations with growth advantage or better adaptation to their microenvironment. The cancer stem cells (CSCs) model postulates –by homology with the hierarchical organization of normal tissues-, the existence of a subset of progenitor cells with the ability to generate the diverse tumor cells. The characteristics of CSCs rely on their "infinite" lifespan, their self renewing, the generation of proliferative progenitors –with limited lifespan- that differentiate and might undergo apoptosis, their ability to disseminate, and their intrinsic resistance to chemotherapies (O'Brien et al., 2007; Cherciu et al., 2014). These two seemingly disparate models, are reconcilable, nevertheless based on the later model, an efficient strategy to cure for cancer implies eradicating CSCs.

Colorectal cancers arise through the stepwise accumulation of genetic alterations leading from normal epithelia to aberrant crypt foci, adenoma, carcinoma and metastatic disease (Fearon and Vogelstein, 1990), and follow three molecular pathways characterized by *i)* chromosomal instability (CIN), *ii)* high microsatellite instability (MSI-H), or *iii)* CpG island methylator phenotype (CIMP).

Colorectal cancers with chromosomal instability account for about 70% of colon cancers (Fearon and Vogelstein, 1990) and are associated with frequent chromosomic alterations (gain chromosome harms 7, 16, 17q 20 and X), and deletions of chromosome harms 1q, 4p, 5q (including *APC, MMC*), 6, 8p, 9q, 17p (including *TP53*), 18q (including *DCC*, *SMAD2/4*) and 22q (Fearon and Vogelstein, 1990; Muleris et al., 1990). These CRC preferentially arise in the left colon.

Tumors MSI-H account for 15% of sporadic CRC, arise more frequently in the proximal colon and are characterized by the deficiency of the DNA mismatch repair system, *e.g.* through biallelic methylation/silencing of the promoter of hMLH1 (Thibodeau et al., 1998). The hypermutated phenotype of these tumors and the neo-antigenic presentation of numerous mutant proteins are associated with a marked leukocyte infiltration, and with a better prognosis in early stages. These mutations are inherited in patients with HNPCC.

The CpG island methylator phenotype (CIMP) is characterized by the methylation of CpG islands in the promoters of certain genes leading to their silencing (Toyota et al., 1999). These tumors are more often located in the proximal colon. Tumors with CIMP-high are frequently associated with MSI as the result of the inactivation of *hMLH1*, as well as $BRAF^{V600E}$ activation, whereas tumors CIMP-low are microsatellite stable (MSS) and bear activating KRAS mutations (Fig. 1). Thus, some overlaps exist between these different tumors types.

At the molecular levels, these 3 types of CRC are associated with a preferential stepwise accumulation of genetic alterations, including the activation/overexpression of a series of (proto)-oncogenes and the deletion/inactivation of tumor suppressor genes. Among them, the phosphoinositide-3-kinase (PI3K)/protein kinase AKT signaling pathway which is important for proliferation of normal and transformed intestinal epithelial cells has been clearly implicated in the progression to the transformed phenotype leading to colorectal carcinoma (Kotelevets et al., 1998, 2001, 2005) (Fig. 1).

Although the genetic defects in these 3 types of CRC differ, it is worth noting that they share similar signaling pathways dysregulation, *e.g. APC* inactivation vs. β-*catenin* and *axin* mutations; *TP53* vs. Bax inactivation; Ki-ras activation vs. BRAF and PIK3CA activation, PTEN inactivation; SMAD2/4 inactivation vs. TGF-βRII, in the CIN and MSI CRC, respectively (Fig. 1).

Taken together, these dysregulations can be classified into one or more of 12 pathways that confer a selective growth advantage, by governing cell survival, cell fate and genome maintenance (Vogelstein et al., 2013)

Besides gene amplification, deletion and mutation, and epigenetic processes, including DNA methylation and histone modifications, other molecular mechanisms have also been linked to neoplastic progression. Alternative splicing allows increasing the biodiversity of proteins with a loss or gain of novel biological functions –as the result of mutation of acceptor or donor sites, or through the selective expression of splicing factors-. In this connection, we previously identified in CRC an activated and novel splice variant of the small GTPase Rac1, we designed Rac1b (Jordan et al., 1999) (Fig. 1). The balance in Rac1/Rac1b levels is regulated by splicing factors, which either induce skipping or favor inclusion of the alternative exon 3b (Goncalves et al., 2009, 2014). The ectopic expression of Rac1b was detected in inflammatory bowel diseases, and in human breast, pancreatic, thyroid, ovarian and lung cancers (Matos et al., 2013; Guo et al., 2015; Mehner et al., 2015; Schnelzer et al., 2000; Silva et al., 2013; Stallings-Mann et al., 2012; Ungefroren et al., 2013; Zhou et al., 2013). Experimental studies performed with transgenic mice revealed that Rac1b cooperated with activated K-Ras to promote lung cancer (Stallings-Mann et al., 2012; Zhou et al., 2013).

Non-coding RNA, *e.g.* miRNA have also been implicated in neoplastic progression. These small RNA molecules (about 22 nucleotides long) negatively control target genes expression posttranscriptionally. Each miRNA is predicted to have several targets, and each mRNA may be regulated by more than one miRNA. Overexpression of some miRNA have been evidenced in CRC, *e.g.* mir21 and mir17-92 which target the mRNA of the PTEN tumor suppressor and of the BIM proapototic protein, respectively (Meng et al., 2007; Poulsen et al., 2014). Interestingly, the accumulation of mir17-92 is mediated by c-myc and β-catenin overexpression, and negatively controlled by APC and TP53 (Diosdado et al., 2009; Li Y. et al., 2016; Poulsen et al., 2014).

3. Cellular interplay in colorectal cancer progression

The invasive phenotype and cancer cell dissemination is postulated to occur through the epithelial–mesenchymal