**Name of journal: *World Journal of Immunology***

**ESPS Manuscript NO: 16510**

**Manuscript Type: Editorial**

**Stem and immune cells in colorectal primary tumour: Number and function of subsets may diagnose metastasis**

Varela-Calviño R *et al*. Stem and immune subsets in CRC

**Rubén Varela-Calviño, Oscar J Cordero**

**Rubén Varela-Calviño, Oscar J Cordero,** Department of Biochemistry and Molecular Biology, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain

**Author contributions:** Varela-Calviño R and Cordero OJ wrote and edited the manuscript.

**Conflict-of-interest** **statement:** The authors declare that they do not have potential financial conflict of interest related to this manuscript.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

**Correspondence to: Oscar J Cordero, PhD,** Department of Biochemistry and Molecular Biology, University of Santiago de Compostela, CIBUS Building, Campus Vida, 15782 Santiago de Compostela, Spain. oscarj.cordero@usc.e

**Telephone:** +34-881-816935

**Received:** January 20, 2015

**Peer-review started:** January 22, 2015

**First decision:** March 6, 2015

**Revised:** May 20, 2015

**Accepted:** July 16, 2015

**Article in press:**

**Published online:**

**Abstract**

An important percentage of colorectal cancer (CRC) patients will develop metastasis, mainly in the liver, even after a successful curative resection. This leads to a very high mortality rate if metastatis is not detected early on. Disseminated cancer cells develop from metastatic stem cells (MetSCs). Recent knowledge has accumulated about these cells particularly in colorectal cancer, so they may now be tracked from the removed primary tumour. This approach could be especially important in prognosis of metastasis because it is becoming clear that metastasis does not particularly rely on testable driver mutations. Among the many traits supporting an epigenetic amplification of cell survival and self-renewal mechanisms of metastatic stem cells, the role of many immune cell populations present in tumour tissues is becoming clear. The amount of tumour-infiltrating lymphocytes (T, B and natural killer cells), dendritic cells and some regulatory populations have already shown prognostic value or to be correlated with disease-free survival time, mainly in immunohistochemistry studies of unique cell populations. Parallel analyses of these immune cell populations together with metastatic stem cells in the primary tumour of patients, with later follow-up data of the patients, will define the usefulness of specific combinations of both immune and MetSCs cell populations. It is expected that these combinations, together to different biomarkers in the form of an immune score, may predict future tumour recurrences, metastases and/or mortality in colorectal cancer. It will also support the future design of improved immunotherapeutic approaches against metastasis.

**Key words:** Colorectal cancer; Metastasis; Stem cells; Immune surveillance; Prognosis; Flow cytometry; Lymphocytes; Dendritic cells; Regulatory cells

© **The Author(s) 2015**. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Metastasis relies on differentiation of some cancer stem cells in the primary tumour niche led by many micro-environmental signals. These signals include the participation of immune cell subsets such as tumour-infiltrating lymphocytes, dendritic cells and regulatory populations.Metastatic stem cells can be identified in the removed primary tumour. The study of the number and function of these immune cell populations in parallel with metastatic stem cells (MetSCs) in the primary tumour, together with follow-up data of patients, will define the usefulness of specific immune and MetSCs cell population combinations. This can be combined with defining new biomarkers as future predictors of tumour recurrences, metastases and/or mortality in colorectal cancer.

Varela-Calviño R, Cordero OJ. Stem and immune cells in colorectal primary tumour: Number and function of subsets may diagnose metastasis.*World J Immunol* 2015; In press

**INTRODUCTION**

Even when a primary tumour has been perfectly removed by surgery, at the moment of diagnosis tumour cells may have disseminated and established themselves in distant locations (metastasis). Metastasis accounts for the vast majority of deaths from cancer. Metastasis is a complex phenomenon developing through several stages, such as the intravasation of cancer cells from the primary tumour, dissemination through the circulation and extravasation in different organs, survival on arrival and settlement into latency, and reactivation, division and colonization of the organ, generating a new macroscopic tumour.

Migrant cancer cells that manage to settle in a distant tissue are known as disseminated tumour cells (DTCs)[1]. However not all disseminated tumour cells are able to generate a new macroscopic tumour and those having such potential are called metastatic stem cells (MetSCs)[2]. The properties that support the survival, self-renewal, dormancy, and reactivation of these MetSCs have been recently reviewed[2], with the most remarkable conclusion being that MetSCs cells have been identified as are cancer stem cells (CSCs).

Most cancers display a hierarchical organization that resembles that of their tissue of origin. CSCs is the only cell type there with long-term self-renewal potential, the microenvironment niche sustaining this potential. They are the phenotypic and functional equivalent of normal stem cells but with the inconvenience of having acquired oncogenic mutations. Both CSCs and non-CSCs can display a migratory behaviour at the invasive front of primary tumours frequently associated with an epithelial to mesenchimal transition. MetSCs may derive from non-stem cell DTCs that reacquire the competence to initiate tumour growth after a period of latency, however this process of phenotypic plasticity is neither totally accepted nor well-understood[2-4]. However, the majority of extravasation and settlement survivors in the host tissue that endow tumour-initiating capacity (*i.e.*, MetSCs) are CSCs[5].

It can be deduced from the data above that MetSCs-cells harbouring the signaling pathways capable to initiate metastasis- already exist in the primary tumour and MetSCs can be tracked from the removed primary tumour. This approach would be specifically important for metastasis diagnosis since it is becoming clear that metastasis does not particularly rely on driver mutations. Therefore, genomic biomarkers are not actually useful for metastasis diagnosis. Environmental and tumour environmental signals do provide the epigenetic amplification for cell survival and self-renewal mechanisms[6].

**METASTATIC STEM CELLS IN COLORECTAL CANCER**

Colorectal cancer is the third most prevalent tumour worldwide. In developed countries, around a 30%-50% of patients who were through a successful curative resection still relapse or develop metastases, mainly in the liver. These patients show a very high mortality rate if those metastases are not detected early[7].

In CRC many lines of evidence support that MetSCs are already present in the primary tumour. A first line of evidence comes from marking of tumour cell populations with lentivirus, which has allowed the clonal analysis of human colorectal cancer cells, showing that metastases arise from primary tumour cells that display long-term self-renewal capacity, are quiescent, and resistant to chemotherapy (*i.e.*, cancer stem cells)[8,9].

A second line of evidence comes from experiments with genetic mouse models. Upon acquiring activating mutations in the Wnt pathway, intestinal stem cells generated adenomas[10]. Another lineage-tracing analysis showed that a stem cell population resembling those present in normal intestinal mucosa not only sustained the long-term growth of these benign lesions[11-13], but also of late stage colorectal cancers and even liver metastases[14-16]. In mice, cell populations characterized by the expression of stem cell markers isolated from human primary tumour samples (CRC and other epithelial tumours) were capable of generating metastasis when transplanted[17-19]. The last line of evidence comes from the clinic, since high expression of adult stem cell markers in primary tumours have been associated with poor prognosis and metastatic relapse[14,15,18,19].

**BIOMARKERS OF METASTATIC STEM CELLS**

An important current question is which stem cell markers should be used for CSCs characterization and whether metastatic stem cells are in fact a CSCs subset that can be tracked using present knowledge.

We have just demonstrated that soluble CD26 levels (sCD26) are a much better serum biomarker for the detection of CRC metastasis or tumour recurrence when compared to other markers in clinical use such as CEA, CA-19.9 or CA-72.4[7] levels. At the same time, others have demonstrated the relationship between the presence of CD26+ cells, detected by immunohistochemistry in primary CRC tumour biopsies and prognosis of metastasis[20]. It is plausible these results are related to the CD26+ CSC population capable of generating metastasis when transplanted in mice[18]. This population comprised CD133+, CD44+ and CD26+ cells isolated from the primary tumour. However, although they majorly encompass the known features of CSCs, they were not the only CSCs present in the primary tumour biopsies. In fact, due to plasticity in CD133 and CD44 expression, these markers do not seem the most appropriate at least as MetSCs markers[21-24]. Another candidates for colorectal cancer MetSCs characterization have been described including CD166, CD29, CD24, Lgr5, EpCAM, ALDH1, CDCP1, CXCR4, CC188[21,23] and ephrin type B receptor 2 (EphB2)[25], although many of these markers are also expressed in normal colonic stem cells (*i.e.*, Lgr5, ALDH1, or CD29) complicating the distinction between CSCs and normal stem cells. Despite this, most of these markers are co-expressed in the primary tumour, so it is expected that a particular biomarker combination can be used to identify MetSCs in colorectal cancers. This will help to understand the function of these cancer stem cells and identify new therapeutic targets as well as to play a significant role in clinical disease management[26]. From our present knowledge, CRC MetSCs should be found among the high-expressing Wnt targets Lgr5++ and EphB2++ cell population[25,27] also co-expressing CD133+ and CXCR4+, markers of a well known metastasic cell population with a recently discovered autofluorescent subcellular compartment[4]. This autofluorescence results from the accumulation of riboflavin in ATP-dependent ABCG2 transporter-coated vesicles exclusively located within the cytoplasm of cells across different human tumour types with cancer stem cell features[4]. It is possible that CD26, intriguingly related to some extent to the CXCR4/SDF-1 axis[28], could also be included among these markers.

**METASTATIC TRAITS IN PRIMARY TUMOURS**

As mentioned above, cell subsets with gene expression signatures to mediate dissemination, survival capability on arrival to distant organs, and entering a dormant state in many cases[2,6-8] before metastatic spreading, have been repeatedly identified in primary tumours[4,25-28]. These traits may be used to predict future relapses before dissemination.

However, (1) there is only a very small percentage of cancer cells with these properties; and (2), these cells are originated by the epigenetic amplification caused by many supporting pathways[2,6]. Little is known about these pathways despite its major clinical importance, since killing latent MetSCs by depriving them of that support seems the most attractive therapeutic approach.

A likely site for selection of metastatic traits in primary tumours is at the invasive front, the intersection of an advancing tumour mass and the surrounding stroma. Cancer cells at the invasive front of primary tumours are exposed to the stresses of invading surrounding tissue, of hypoxia, and of the immune surveillance. This complex milieu includes cancer-associated fibroblasts (CAFs), newly generated blood vessels[29], tumor-associated macrophages, myeloid progenitor cells, and blood platelets. Various stromal cell types produce cytokines such as Wnt, Notch, tumour necrosis factor- alpha alpha(TNF-αa), transforming growth factor-beta (TGF-a), hepatocyte growth factor and hedgehog, which support the survival and fitness of CSCs[16,30-32]. Under selective pressure, these signals skew the heterogeneous cancer cell population towards a preponderance of clones primed for survival, self-renewal, invasiveness, migration, and the stress of infiltrating distant tissues (*i.e.*, future MetSCs).

In fact, it seems that when the stroma of a primary tumor is rich in cells and signals resembling those of a particular distant tissue, cancer cell clones selected in this primary tumour could be primed to thrive in that particular tissue[2,29,30]. For example, cells and signals in a colorectal gut tumour resembling the liver environment will induce metastasis of this CRC in the liver[33].

At the same time, some already cited tumour-derived soluble factors together with other signals such as VEGF, SDF-1, IL-10, and enzymes such as indoleamine 2, 3-dioxygenase (IDO) or cyclooxygenase-2 (COX-2), or the adenosine pathway, are well known factors responsible for the expansion of induced-T regulatory T cells (iTreg) in tumour-bearing hosts[34-37] as well as for inducing the accumulation of immature dendritic cells (iDCs), which in turn promote the expansion of iTreg[38]. Both phenotypically and functionally, iTreg cells are distinct from natural Treg (nTreg) and accumulate both in tissues and peripheral blood of cancer patients. These iTreg are presumably responsible for the suppression of anti-tumour functions of immune cells migrating to the tumour site, thus promoting tumour escape from the host immune response[35].

**IMMUNE SURVEILLANCE IN COLORECTAL CANCER**

From the point of view of the three immune hallmarks of cancer stating that tumours (1) are able to thrive in a chronically inflamed microenvironment; (2) can evade immunorecognition; and (3) are able to suppress immune reactivity[39], CRC is particularly known for the many evidences connecting tumourigenesis and inflammation, such as the decreased incidence of tumours in individuals under non-steroidal anti-inflammatory drug treatment, the increased incidence of tumours in overweight patients, and its relationship with commensal bacteria. We have reviewed recently these facts altogether affecting inflammation both locally and systemically[40].

According to this activation of the immune system, cells of the innate immune system such as neutrophils[41], macrophages[42], natural killer (NK) cells[43] or DCs[44] as well as cells of the adaptive immune system such as CD4+ helper and CD8+ cytotoxic T lymphocytes (CTLs)[45,46] accumulate in sites of CRC development. Although immune cells release inflammatory mediators (see above) with proangiogenic and prometastasic effects[47] to the reactive stroma, at the same time tumour-infiltrating lymphocytes (TILs) in CRC have been shown to inhibit tumour growth and are associated with improved prognosis[46-52].

The concept of cancer immunoediting[53] has been divided into three phases namely elimination, equilibrium and escape[54]. In the elimination phase or cancer immunosurveillance, immune cells detect and eliminate transformed cells but this elimination could be incomplete in which case some tumour cells remain either dormant or continue to evolve accumulating further changes that can modulate the expression of tumour-associated antigens (TAAs) or other factors that increase their fitness. During this time the immune system still exerts a selective pressure eliminating some transformed clones but if this elimination is again incomplete, the process results in the selection of tumour cell variants (MetSCs among them) which are able to resist, avoid or suppress the anti-tumor response, leading to the escape phase[54].

It has been shown that CRC induces an immunosuppression state, marked by reduced secretion in patients of several cytokines such as IFN-γγg or TNF-a by monocytes/macrophages. As this immunosuppression was reversible after resection of the affected tissue[55], this data held the promise of immunologically targeting tumour cells, provided the mechanisms of immune escape and tumour-induced immune suppression are overcome.

**T CELLS**

As previously mentioned, human CRC tissue is infiltrated by a variety of immune cells often in the margins of the transformed tissue, in the invasive front. Several studies have characterized the lymphocyte infiltration of CRC and confirmed the concept of prognostic impact of these TILs[45,56]. In most cases, the lymphocytes infiltrating the cancer tissue, and most frequently the area along the invasive margin, are either CD4+ and/or CD8+ T cells[57].

Despite their low numbers, CD8+ T lymphocytes infiltrating the neoplastic epithelium are positively correlated with longer disease-free survival time[52,56-58] and in fact, the density of T CD8+ and CD45+ lymphocyte infiltration was recently shown to have a better prognostic value than the classic tumor node metastasis classification factor[59]. Previous data have shown that these TILs have antitumor activity[60,61], and some TAAs have been identified as potential targets of cytotoxic CD8+ T lymphocyte responses[60-62]. Later, T cell responses against mutated normal antigens such as those of the microsatellite instable (MSI) subgroup of CRC or the small subgroup of tumours with no signs of MSI but also with high mutational load were detected[50,63,64]. Coherently, the microsatellite instability-high (MSI-H) phenotype present in 15% of early CRC confers good prognosis[50,65]. Therefore these so-called tumour-specific somatic mutations are potentially the best targets for adoptive T cell therapy, although there are many open questions like how many somatic mutations create suitable epitopes[66]. However, at the same time these tumours have clearly adapted to this immune pressure because many CRCs express no or reduced levels of HLA-I[67-69]. Although this is a classical mechanism of transformed cells to avoid the host immune response[70,71], there are conflicting results regarding CRC expression of HLA class I antigens as associated with poor prognosis[72], probably because NK cells are important effectors in the anti-tumour response against CRC (see below).

During an immune response CD4+ T lymphocytes can differentiate into two broad phenotypic subtypes: T helper 1 (Th1) or Th2[73,74]. These two different subtypes secrete different types of cytokines, and consequently activate different types of immune responses. Th1 lymphocytes secrete IFN-gγ and TNF-a, which produce the activation of CTLs, NK cells, macrophages and monocytes, all of which contribute to a cellular immune response that is effective against tumour cells. However, Th2 lymphocytes secrete a different set of cytokines such as IL-4, IL-5, IL-10 or IL-13, all of which deviate the response to a humoral immune response, and this kind of immune response is less effective at eliminating cancer cells[73-75]. A shift towards a Th2 response has been shown in CRC patients, with reduced levels of Th1 cytokines and normal or elevated levels of Th2 cytokines, an imbalance that becomes more significant the further the disease progresses[76-78], with levels of the Th1 cytokines having a prognostic value in terms of patient survival [73].

The mechanism through which CRC cells can shift the T cell immune response could be due in part to the secretion of cytokines that inhibit the development of Th1 responses, such as TGF-b and IL-10, either by the CRC cells themselves or CAFs[79]. Among the roles assigned to TGF-a in cancer development[79-85], it has been cited the inhibition of T lymphocyte proliferation and differentiation preventing naïve T cells from acquiring effector functions[86] and the inhibition of the ability of TILs to kill cancer cells as well as tumour-specific CD8+ cytotoxic responses[87], although recently discovered stromal factors such as tumour-derived exosomes carrying death receptor ligands directly contribute to apoptosis of activated effector CD8+ T cells[88]. IL-10 immunosuppresses TILs[89] but this immunosuppressive effect is mainly indirect and mediated by DCs and Treg lymphocytes (see below)[90,91].

In addition, although the role of other T helper populations, Th17 and Th22, in the development of CRC is still unclear, it seems that decreased Th17 and Th22 responses are associated with the development of CRC[92].

**B CELLS**

Many of the TAAs identified in CRC so far, potential targets of cytotoxic CD8+ T lymphocyte responses, has been done by the identification of auto-antibodies present in the plasma of cancer patients compared to healthy donors, and although the clinical significance of those serologically-defined antigens still have to be demonstrated, several are attractive candidates for cancer vaccines[60]. Interestingly, antibody responses against some TAAs correlate with CD8+ responses in those patients[61,62], supporting the idea that the immune response taking place in CRC patients requires coordinated CD4+, CD8+ and B cell responses, turning Th2 anti-tumour responses a not so negative factor as previously supposed[73]. However, tumour-infiltrating CD20+ B cells (TIL-B) have being poorly investigated despite their described positive prognostic value[93]. Engagement of tumour-reactive B cells may be an important condition for generating potent, long-term T cell responses against cancer[94].

**DENDRITIC CELLS**

DCs are key antigen-presenting cells that play a central role in the induction of immune responses including anti-tumour responses[95,96]. It has been shown that CRC patients have DCs infiltrating the tumour mass or the surrounding tissue forming clusters with T lymphocytes[97] and that this infiltration seems to correlate with a better prognosis[98,99]. In fact, activated and matured DCs induce an antigen-specific response leading to T cell proliferation and differentiation into helper and effector lymphocytes[100].

However, CRC tumour cells are able to impair the function of these cells. *In vivo* tumour-infiltrating DCs show an immature phenotype[101] and iDCs presenting self-antigens to both CD4+ and CD8+ T cells induce tolerance in those lymphocytes[102,103]. In this direction, tissue culture media from CRC explants inhibits DC maturation with reduced levels of CD54, CD86, HLA-DR and CD83, and induces IL-10 secretion while inhibiting secretion of IL-12p70, factors that inhibit Th1 immune responses and probably protect the tumour from a potent immune response[104]. Moreover, as mentioned before, iDCs correlate with infiltration and the expansion of iTregs [35, 103].

**NK CELLS**

NK cells play a major role in the immune response to CRC[59] and are a prognostic factor[105].

NK cells are typically defective in infiltrating solid tumors with only 30% of patients showing NK infiltration and with only a 9% with more than four NK cells, as it has been shown in a large cohort[106]. Tumour cells has several mechanisms to inhibit recruitment and activation of NK cells[107-109], but this fact does not have a direct effect on tumor progression *per se*[107] probably explaining why the presence of NK cells in combination with CD4+ T lymphocytes in colorectal tumours had no detectable effect on the clinical course of the disease[43,106].

However, in CRC the infiltration of both NK cells and CD8+ T cells was associated with prolonged patient survival in the same study, suggesting NK-CD8+ cell crosstalk in the tumor microenvironment[106]. These data agree with the fact that *ex vivo* activation and expansion of both NK and CTLs followed by their intraveneous infusion in patients with stage IV colon cancer improved their quality of life[110], or the fact that one of the mechanisms of action of cetuximab, a monoclonal antibody against the epidermal growth factor receptor widely used for the treatment of metastatic colorectal cancer (mCRC), is antibody-dependent cell-mediated cytotoxicity, triggered by Fc-gamma-R on NK cells[59,111].

Of the utmost importance is the fact that NK cells play a crucial role in preventing recurrence[112] probably because they are able to target CSCs/MetSCs[113].

**REGULATORY CELLS**

Treg cells characterized by the expression of CD25 and the transcription factor Foxp3 are critical for the prevention of autoimmunity and the regulation of immune responses to foreign and self-antigens[114]. Adaptive iTreg, a distinct population from nTreg, accumulate in tissues and the peripheral blood of cancer patients. In many of those human cancers high densities of such Tregs in the tumor correlates with poor disease outcome[115].

However, they are associated with an improved survival rate of CRC patients[115,116], or other carcinoma with prominent inflammatory infiltrates (*i.e.*, certain types of breast cancer), despite iTreg contrasted functionality[117,118]. A hypothesis has been put forward to explain this apparent contradiction indicating that those Foxp3+ Tregs infiltrating the tumour mass were already in the healthy colorectal tissue to suppress excessive inflammation and immune responses resulting from the commensal microflora[103,119].

It has been hypothesized that these cells posses a contextual plasticity controlled and driven by the tissue microenvironment[103]. The main question is which factors or signals in the microenvironment regulate Treg functions thereby preventing adverse effects of chronic inflammation or autoimmunity[120]. It seems that the cellular content of the colorectal cancer infiltrate do that by silencing the tolerogenic pathway of plasmacytoid DCs[121]. These cells, different to myeloid DCs, additionally promote tolerance and Treg differentiation and suppressor functions in the solid tumour presumably *via* the Nrp-1/semaphorin-4ª pathway (plasmacytoid DCs are one of the major sources of semaphorin-4ª), and the infiltrate would block this pathway[120]. Thus, it is important monitoring not only for the frequency but also for the functionality of iTreg in cancer.

In addition, the presence of other regulatory populations such as natural killer T (NKT) cells or Bregs can not be excluded since the nature of the regulatory cell types that dominate in any given tumour is not totally understood[122,123]. The role played by regulatory type I and II NKT cells has been studied in syngeneic mice models of colorectal and renal cancer. In those models, having both type I and II NKT cells or neither of them, Treg depletion was sufficient to protect against tumour outgrowth, however in those mice lacking only type I NKT cells, Treg blockade was insufficient to protect mice pointing to an important role played by type II NKT cells in suppressing tumour growth[123].

**HYPOTHESIS**

An important reduction in the level of serum sCD26 in patients with non-metastasic CRC makes sCD26 a promising candidate for a future serum screening test[125]. We have previously suggested that these altered levels in CRC could be due to alterations in the number or frequency of lymphocyte populations expressing this biomarker[7,124],

We pretend to analyze by flow cytometry the expression of CD26 in the different leukocyte cell populations mentioned above that could be identified in primary CRC tissue biopsies. These analyses will be combined in parallel with the analysis of the known markers for MetSCs in cells of the same tissue[48,126]. All this information, together with the follow-up of the patients for up to 5 years, will help to define the usefulness of different cell population combinations, both immune and CSCs, and/or biomarkers. These combinations will assemble an immune score[59,101] that functions as predictor of future tumour recurrences, metastases and/or mortality in CRC. At the same time, this increased knowledge will support a better design of future immunotherapeutic approaches against metastasis.

Moreover, from a methodological point of view, the use of flow cytometry allows very potent qualitative and quantitative multiparametric analyses, contributing with new information to classical and modern[57,58,127] anatomopathological studies where no *in situ* information is lost.

**REFERENCES**

1 **Pantel K**, Brakenhoff RH, Brandt B. Detection, clinical relevance and specific biological properties of disseminating tumour cells. *Nat Rev Cancer* 2008; **8**: 329-340 [PMID: 18404148 DOI: 10.1038/nrc2375]

2 **Oskarsson T**, Batlle E, Massagué J. Metastatic stem cells: sources, niches, and vital pathways. *Cell Stem Cell* 2014; **14**: 306-321 [PMID: 24607405 DOI: 10.1016/j.stem.2014.02.002]

3 **Liu S**, Cong Y, Wang D, Sun Y, Deng L, Liu Y, Martin-Trevino R, Shang L, McDermott SP, Landis MD, Hong S, Adams A, D'Angelo R, Ginestier C, Charafe-Jauffret E, Clouthier SG, Birnbaum D, Wong ST, Zhan M, Chang JC, Wicha MS. Breast cancer stem cells transition between epithelial and mesenchymal states reflective of their normal counterparts. *Stem Cell Reports* 2014; **2**: 78-91 [PMID: 24511467 DOI: 10.1016/j.stemcr.2013.11.009]

4 **Miranda-Lorenzo I**, Dorado J, Lonardo E, Alcala S, Serrano AG, Clausell-Tormos J, Cioffi M, Megias D, Zagorac S, Balic A, Hidalgo M, Erkan M, Kleeff J, Scarpa A, Sainz B, Heeschen C. Intracellular autofluorescence: a biomarker for epithelial cancer stem cells. *Nat Methods* 2014; **11**: 1161-1169 [PMID: 25262208 DOI: 10.1038/nmeth.3112]

5 **Driessens G**, Beck B, Caauwe A, Simons BD, Blanpain C. Defining the mode of tumour growth by clonal analysis. *Nature* 2012; **488**: 527-530 [PMID: 22854777 DOI: 10.1038/nature11344]

6 **Vanharanta S**, Massagué J. Origins of metastatic traits. *Cancer Cell* 2013; **24**: 410-421 [PMID: 24135279 DOI: 10.1016/j.ccr.2013.09.007]

7 **De Chiara L**, Rodríguez-Piñeiro AM, Cordero OJ, Vázquez-Tuñas L, Ayude D, Rodríguez-Berrocal FJ, de la Cadena MP. Postoperative serum levels of sCD26 for surveillance in colorectal cancer patients. *PLoS One* 2014; **9**: e107470 [PMID: 25210927 DOI: 10.1371/journal.pone.0107470]

8 **Dieter SM**, Ball CR, Hoffmann CM, Nowrouzi A, Herbst F, Zavidij O, Abel U, Arens A, Weichert W, Brand K, Koch M, Weitz J, Schmidt M, von Kalle C, Glimm H. Distinct types of tumor-initiating cells form human colon cancer tumors and metastases. *Cell Stem Cell* 2011; **9**: 357-365 [PMID: 21982235 DOI: 10.1016/j.stem.2011.08.010]

9 **Kreso A**, O'Brien CA, van Galen P, Gan OI, Notta F, Brown AM, Ng K, Ma J, Wienholds E, Dunant C, Pollett A, Gallinger S, McPherson J, Mullighan CG, Shibata D, Dick JE. Variable clonal repopulation dynamics influence chemotherapy response in colorectal cancer. *Science* 2013; **339**: 543-548 [PMID: 23239622 DOI: 10.1126/science.1227670]

10 **Barker N**, Ridgway RA, van Es JH, van de Wetering M, Begthel H, van den Born M, Danenberg E, Clarke AR, Sansom OJ, Clevers H. Crypt stem cells as the cells-of-origin of intestinal cancer. *Nature* 2009; **457**: 608-611 [PMID: 19092804 DOI: 10.1038/nature07602]

11 **Kozar S**, Morrissey E, Nicholson AM, van der Heijden M, Zecchini HI, Kemp R, Tavaré S, Vermeulen L, Winton DJ. Continuous clonal labeling reveals small numbers of functional stem cells in intestinal crypts and adenomas. *Cell Stem Cell* 2013; **13**: 626-633 [PMID: 24035355 DOI: 10.1016/j.stem.2013.08.001]

12 **Nakanishi Y**, Seno H, Fukuoka A, Ueo T, Yamaga Y, Maruno T, Nakanishi N, Kanda K, Komekado H, Kawada M, Isomura A, Kawada K, Sakai Y, Yanagita M, Kageyama R, Kawaguchi Y, Taketo MM, Yonehara S, Chiba T. Dclk1 distinguishes between tumor and normal stem cells in the intestine. *Nat Genet* 2013; **45**: 98-103 [PMID: 23202126 DOI: 10.1038/ng.248]

13 **Schepers AG**, Snippert HJ, Stange DE, van den Born M, van Es JH, van de Wetering M, Clevers H. Lineage tracing reveals Lgr5+ stem cell activity in mouse intestinal adenomas. *Science* 2012; **337**: 730-735 [PMID: 22855427 DOI: 10.1038/ng.2481]

14 **Dalerba P**, Kalisky T, Sahoo D, Rajendran PS, Rothenberg ME, Leyrat AA, Sim S, Okamoto J, Johnston DM, Qian D, Zabala M, Bueno J, Neff NF, Wang J, Shelton AA, Visser B, Hisamori S, Shimono Y, van de Wetering M, Clevers H, Clarke MF, Quake SR. Single-cell dissection of transcriptional heterogeneity in human colon tumors. *Nat Biotechnol* 2011; **29**: 1120-1127 [PMID: 22081019 DOI: 10.1038/nbt.2038]

15 **Merlos-Suárez A**, Barriga FM, Jung P, Iglesias M, Céspedes MV, Rossell D, Sevillano M, Hernando-Momblona X, da Silva-Diz V, Muñoz P, Clevers H, Sancho E, Mangues R, Batlle E. The intestinal stem cell signature identifies colorectal cancer stem cells and predicts disease relapse. *Cell Stem Cell* 2011; **8**: 511-524 [PMID: 21419747 DOI: 10.1016/j.stem.2011.02.020]

16 **Vermeulen L**, De Sousa E Melo F, van der Heijden M, Cameron K, de Jong JH, Borovski T, Tuynman JB, Todaro M, Merz C, Rodermond H, Sprick MR, Kemper K, Richel DJ, Stassi G, Medema JP. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nat Cell Biol* 2010; **12**: 468-476 [PMID: 20418870 DOI: 10.1038/ncb2048]

17 **Malanchi I**, Santamaria-Martínez A, Susanto E, Peng H, Lehr HA, Delaloye JF, Huelsken J. Interactions between cancer stem cells and their niche govern metastatic colonization. *Nature* 2012; **481**: 85-89 [PMID: 22158103 DOI: 10.1038/nature10694]

18 **Pang R**, Law WL, Chu AC, Poon JT, Lam CS, Chow AK, Ng L, Cheung LW, Lan XR, Lan HY, Tan VP, Yau TC, Poon RT, Wong BC. A subpopulation of CD26+ cancer stem cells with metastatic capacity in human colorectal cancer. *Cell Stem Cell* 2010; **6**: 603-615 [PMID: 20569697 DOI: 10.1016/j.stem.2010.04.001]

19 **Pece S**, Tosoni D, Confalonieri S, Mazzarol G, Vecchi M, Ronzoni S, Bernard L, Viale G, Pelicci PG, Di Fiore PP. Biological and molecular heterogeneity of breast cancers correlates with their cancer stem cell content. *Cell* 2010; **140**: 62-73 [PMID: 20074520 DOI: 10.1016/j.cell.2009.12.007]

20 **Lam CS**, Cheung AH, Wong SK, Wan TM, Ng L, Chow AK, Cheng NS, Pak RC, Li HS, Man JH, Yau TC, Lo OS, Poon JT, Pang RW, Law WL. Prognostic significance of CD26 in patients with colorectal cancer. *PLoS One* 2014; **9**: e98582 [PMID: 24870408 DOI: 10.1371/journal.pone.0098582]

21 **Hsu CS**, Tung CY, Yang CY, Lin CH. Response to stress in early tumor colonization modulates switching of CD133-positive and CD133-negative subpopulations in a human metastatic colon cancer cell line, SW620. *PLoS One* 2013; **8**: e61133 [PMID: 23577199 DOI: 10.1371/journal.pone.0061133]

22 **Ren F**, Sheng WQ, Du X. CD133: a cancer stem cells marker, is used in colorectal cancers. *World J Gastroenterol* 2013; **19**: 2603-2611 [PMID: 23674867 DOI: 10.3748/wjg.v19.i17.2603]

23 **Pitule P**, Cedikova M, Daum O, Vojtisek J, Vycital O, Hosek P, Treska V, Hes O, Kralickova M, Liska V. Immunohistochemical detection of cancer stem cell related markers CD44 and CD133 in metastatic colorectal cancer patients. *Biomed Res Int* 2014; **2014**: 432139 [PMID: 24864242 DOI: 10.1155/2014/432139]

24 **Rowehl RA**, Burke S, Bialkowska AB, Pettet DW, Rowehl L, Li E, Antoniou E, Zhang Y, Bergamaschi R, Shroyer KR, Ojima I, Botchkina GI. Establishment of highly tumorigenic human colorectal cancer cell line (CR4) with properties of putative cancer stem cells. *PLoS One* 2014; **9**: e99091 [PMID: 24921652 DOI: 10.1371/journal.pone.0099091]

25 **Jung P**, Sato T, Merlos-Suárez A, Barriga FM, Iglesias M, Rossell D, Auer H, Gallardo M, Blasco MA, Sancho E, Clevers H, Batlle E. Isolation and in vitro expansion of human colonic stem cells. *Nat Med* 2011; **17**: 1225-1227 [PMID: 21892181 DOI: 10.1038/nm.2470]

26 **Li CJ**, Zhang X, Fan GW. Updates in colorectal cancer stem cell research. *J Cancer Res Ther* 2014; **10** Suppl: 233-239 [PMID: 25693926 DOI: 10.4103/0973-1482.151449]

27 **Kemper K**, Prasetyanti PR, De Lau W, Rodermond H, Clevers H, Medema JP. Monoclonal antibodies against Lgr5 identify human colorectal cancer stem cells. *Stem Cells* 2012; **30**: 2378-2386 [PMID: 22969042 DOI: 10.1002/stem.1233]

28 **Havre PA**, Abe M, Urasaki Y, Ohnuma K, Morimoto C, Dang NH. CD26 expression on T cell lines increases SDF-1-alpha-mediated invasion. *Br J Cancer* 2009; **101**: 983-991 [PMID: 19654580 DOI: 10.1038/sj.bjc.6605236]

29 **Joyce JA**, Pollard JW. Microenvironmental regulation of metastasis. *Nat Rev Cancer* 2009; **9**: 239-252 [PMID: 19279573 DOI: 10.1038/nrc2618]

30 **Takebe N**, Harris PJ, Warren RQ, Ivy SP. Targeting cancer stem cells by inhibiting Wnt, Notch, and Hedgehog pathways. *Nat Rev Clin Oncol* 2011; **8**: 97-106 [PMID: 21151206 DOI: 10.1038/nrclinonc.2010.196]

31 **Anido J**, Sáez-Borderías A, Gonzàlez-Juncà A, Rodón L, Folch G, Carmona MA, Prieto-Sánchez RM, Barba I, Martínez-Sáez E, Prudkin L, Cuartas I, Raventós C, Martínez-Ricarte F, Poca MA, García-Dorado D, Lahn MM, Yingling JM, Rodón J, Sahuquillo J, Baselga J, Seoane J. TGF-β Receptor Inhibitors Target the CD44(high)/Id1(high) Glioma-Initiating Cell Population in Human Glioblastoma. *Cancer Cell* 2010; **18**: 655-668 [PMID: 21156287 DOI: 10.1016/j.ccr.2010.10.023]

32 **Scheel C**, Eaton EN, Li SH, Chaffer CL, Reinhardt F, Kah KJ, Bell G, Guo W, Rubin J, Richardson AL, Weinberg RA. Paracrine and autocrine signals induce and maintain mesenchymal and stem cell states in the breast. *Cell* 2011; **145**: 926-940 [PMID: 21663795 DOI: 10.1016/j.cell.2011.04.029]

33 **Zhang XH**, Jin X, Malladi S, Zou Y, Wen YH, Brogi E, Smid M, Foekens JA, Massagué J. Selection of bone metastasis seeds by mesenchymal signals in the primary tumor stroma. *Cell* 2013; **154**: 1060-1073 [PMID: 23993096 DOI: 10.1016/j.cell.2013.07.036]

34 **Chen W**, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, McGrady G, Wahl SM. Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J Exp Med* 2003; **198**: 1875-1886 [PMID: 14676299]

35 **Zou W**. Immunosuppressive networks in the tumour environment and their therapeutic relevance. *Nat Rev Cancer* 2005; **5**: 263-274 [PMID: 15776005 DOI: 10.1038/nrc1586]

36 **Yan M**, Jene N, Byrne D, Millar EK, O'Toole SA, McNeil CM, Bates GJ, Harris AL, Banham AH, Sutherland RL, Fox SB. Recruitment of regulatory T cells is correlated with hypoxia-induced CXCR4 expression, and is associated with poor prognosis in basal-like breast cancers. *Breast Cancer Res* 2011; **13**: R47 [PMID: 21521526 DOI: 10.1186/bcr2869]

37 **Popple A**, Durrant LG, Spendlove I, Rolland P, Scott IV, Deen S, Ramage JM. The chemokine, CXCL12, is an independent predictor of poor survival in ovarian cancer. *Br J Cancer* 2012; **106**: 1306-1313 [PMID: 22415233 DOI: 10.1038/bjc.2012.49]

38 **Chung DJ**, Rossi M, Romano E, Ghith J, Yuan J, Munn DH, Young JW. Indoleamine 2,3-dioxygenase-expressing mature human monocyte-derived dendritic cells expand potent autologous regulatory T cells. *Blood* 2009; **114**: 555-563 [PMID: 19465693]

39 **Cavallo F**, De Giovanni C, Nanni P, Forni G, Lollini PL. 2011: the immune hallmarks of cancer. *Cancer Immunol Immunother* 2011; **60**: 319-326 [PMID: 21267721 DOI: 10.1007/s00262-010-0968-0]

40 **Varela-Calviño R**, Cordero OJ. Immunology and immunotherapy of colorectal cancer. In: “Cancer Immunology. Cancer Immunotherapy for Organ-Specific Tumors”. Rezaei N, editor. Springer-Verlag, 2015

41 **Rao HL**, Chen JW, Li M, Xiao YB, Fu J, Zeng YX, Cai MY, Xie D. Increased intratumoral neutrophil in colorectal carcinomas correlates closely with malignant phenotype and predicts patients' adverse prognosis. *PLoS One* 2012; **7**: e30806 [PMID: 22295111 DOI: 10.1371/journal.pone.0030806]

42 **Algars A**, Irjala H, Vaittinen S, Huhtinen H, Sundström J, Salmi M, Ristamäki R, Jalkanen S. Type and location of tumor-infiltrating macrophages and lymphatic vessels predict survival of colorectal cancer patients. *Int J Cancer* 2012; **131**: 864-873 [PMID: 21952788 DOI: 10.1002/ijc.26457]

43 **Papanikolaou IS**, Lazaris AC, Apostolopoulos P, Kavantzas N, Papas MG, Mavrogiannis C, Patsouris ES, Archimandritis A. Tissue detection of natural killer cells in colorectal adenocarcinoma. *BMC Gastroenterol* 2004; **4**: 20 [PMID: 15363095 DOI: 10.1186/1471-230X-4-20]

44 **Nagorsen D**, Voigt S, Berg E, Stein H, Thiel E, Loddenkemper C. Tumor-infiltrating macrophages and dendritic cells in human colorectal cancer: relation to local regulatory T cells, systemic T-cell response against tumor-associated antigens and survival. *J Transl Med* 2007; **5**: 62 [PMID: 18047662 DOI: 10.1186/1479-5876-5-62]

45 **Koch M**, Beckhove P, Op den Winkel J, Autenrieth D, Wagner P, Nummer D, Specht S, Antolovic D, Galindo L, Schmitz-Winnenthal FH, Schirrmacher V, Büchler MW, Weitz J. Tumor infiltrating T lymphocytes in colorectal cancer: Tumor-selective activation and cytotoxic activity in situ. *Ann Surg* 2006; **244**: 986-992; discussion 992-993 [PMID: 17122624]

46 **Naito Y**, Saito K, Shiiba K, Ohuchi A, Saigenji K, Nagura H, Ohtani H. CD8+ T cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer. *Cancer Res* 1998; **58**: 3491-3494 [PMID: 9721846]

47 **Coussens LM**, Werb Z. Inflammation and cancer. *Nature* 2002; **420**: 860-867 [PMID: 12490959 DOI: 10.1038/nature01322]

48 **Pagès F**, Berger A, Camus M, Sanchez-Cabo F, Costes A, Molidor R, Mlecnik B, Kirilovsky A, Nilsson M, Damotte D, Meatchi T, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Galon J. Effector memory T cells, early metastasis, and survival in colorectal cancer. *N Engl J Med* 2005; **353**: 2654-2666 [PMID: 16371631 DOI: 10.1056/NEJMoa051424]

49 **Diederichsen AC**, Hjelmborg Jv, Christensen PB, Zeuthen J, Fenger C. Prognostic value of the CD4+/CD8+ ratio of tumour infiltrating lymphocytes in colorectal cancer and HLA-DR expression on tumour cells. *Cancer Immunol Immunother* 2003; **52**: 423-428 [PMID: 12695859]

50 **Banerjea A**, Bustin SA, Dorudi S. The immunogenicity of colorectal cancers with high-degree microsatellite instability. *World J Surg Oncol* 2005; **3**: 26 [PMID: 15890075 DOI: 10.1186/1477-7819-3-26]

51 **Ropponen KM**, Eskelinen MJ, Lipponen PK, Alhava E, Kosma VM. Prognostic value of tumour-infiltrating lymphocytes (TILs) in colorectal cancer. *J Pathol* 1997; **182**: 318-324 [PMID: 9349235]

52 **Reissfelder C**, Stamova S, Gossmann C, Braun M, Bonertz A, Walliczek U, Grimm M, Rahbari NN, Koch M, Saadati M, Benner A, Büchler MW, Jäger D, Halama N, Khazaie K, Weitz J, Beckhove P. Tumor-specific cytotoxic T lymphocyte activity determines colorectal cancer patient prognosis. *J Clin Invest* 2015; **125**: 739-751 [PMID: 25562322 DOI: 10.1172/JCI74894]

53 **Dunn GP**, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. *Nat Immunol* 2002; **3**: 991-998 [PMID: 12407406 DOI: 10.1038/ni1102-991]

54 **Dunn GP**, Old LJ, Schreiber RD. The three Es of cancer immunoediting. *Annu Rev Immunol* 2004; **22**: 329-360 [PMID: 15032581 DOI: 10.1146/annurev.immunol.22.012703.104803]

55 **Heriot AG**, Marriott JB, Cookson S, Kumar D, Dalgleish AG. Reduction in cytokine production in colorectal cancer patients: association with stage and reversal by resection. *Br J Cancer* 2000; **82**: 1009-1012 [PMID: 10737381 DOI: 10.1054/bjoc.1999.1034]

56 **Ogino S**, Nosho K, Irahara N, Meyerhardt JA, Baba Y, Shima K, Glickman JN, Ferrone CR, Mino-Kenudson M, Tanaka N, Dranoff G, Giovannucci EL, Fuchs CS. Lymphocytic reaction to colorectal cancer is associated with longer survival, independent of lymph node count, microsatellite instability, and CpG island methylator phenotype. *Clin Cancer Res* 2009; **15**: 6412-6420 [PMID: 19825961 DOI: 10.1158/1078-0432.CCR-09-1438]

57 **Menon AG**, Janssen-van Rhijn CM, Morreau H, Putter H, Tollenaar RA, van de Velde CJ, Fleuren GJ, Kuppen PJ. Immune system and prognosis in colorectal cancer: a detailed immunohistochemical analysis. *Lab Invest* 2004; **84**: 493-501 [PMID: 14968119 DOI: 10.1038/labinvest.3700055]

58 **Chiba T**, Ohtani H, Mizoi T, Naito Y, Sato E, Nagura H, Ohuchi A, Ohuchi K, Shiiba K, Kurokawa Y, Satomi S. Intraepithelial CD8+ T-cell-count becomes a prognostic factor after a longer follow-up period in human colorectal carcinoma: possible association with suppression of micrometastasis. *Br J Cancer* 2004; **91**: 1711-1717 [PMID: 15494715 DOI: 10.1038/sj.bjc.6602201]

59 **Pernot S**, Terme M, Voron T, Colussi O, Marcheteau E, Tartour E, Taieb J. Colorectal cancer and immunity: what we know and perspectives. *World J Gastroenterol* 2014; **20**: 3738-3750 [PMID: 24833840 DOI: 10.3748/wjg.v20.i14.3738]

60 **Nagorsen D**, Keilholz U, Rivoltini L, Schmittel A, Letsch A, Asemissen AM, Berger G, Buhr HJ, Thiel E, Scheibenbogen C. Natural T-cell response against MHC class I epitopes of epithelial cell adhesion molecule, her-2/neu, and carcinoembryonic antigen in patients with colorectal cancer. *Cancer Res* 2000; **60**: 4850-4854 [PMID: 10987297]

61 **Jäger E**, Nagata Y, Gnjatic S, Wada H, Stockert E, Karbach J, Dunbar PR, Lee SY, Jungbluth A, Jäger D, Arand M, Ritter G, Cerundolo V, Dupont B, Chen YT, Old LJ, Knuth A. Monitoring CD8 T cell responses to NY-ESO-1: correlation of humoral and cellular immune responses. *Proc Natl Acad Sci USA* 2000; **97**: 4760-4765 [PMID: 10781081 DOI: 10.1073/pnas.97.9.4760]

62 **Jäger E**, Gnjatic S, Nagata Y, Stockert E, Jäger D, Karbach J, Neumann A, Rieckenberg J, Chen YT, Ritter G, Hoffman E, Arand M, Old LJ, Knuth A. Induction of primary NY-ESO-1 immunity: CD8+ T lymphocyte and antibody responses in peptide-vaccinated patients with NY-ESO-1+ cancers. *Proc Natl Acad Sci USA* 2000; **97**: 12198-12203 [PMID: 11027314 DOI: 10.1073/pnas.220413497]

63 **Bauer K**, Nelius N, Reuschenbach M, Koch M, Weitz J, Steinert G, Kopitz J, Beckhove P, Tariverdian M, von Knebel Doeberitz M, Kloor M. T cell responses against microsatellite instability-induced frameshift peptides and influence of regulatory T cells in colorectal cancer. *Cancer Immunol Immunother* 2013; **62**: 27-37 [PMID: 22729559 DOI: 10.1007/s00262-012-1303-8]

64 [**Nishimura Y**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Nishimura%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=25726868), [Tomita Y](http://www.ncbi.nlm.nih.gov/pubmed/?term=Tomita%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=25726868), [Yuno A](http://www.ncbi.nlm.nih.gov/pubmed/?term=Yuno%20A%5BAuthor%5D&cauthor=true&cauthor_uid=25726868), [Yoshitake Y](http://www.ncbi.nlm.nih.gov/pubmed/?term=Yoshitake%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=25726868), [Shinohara M](http://www.ncbi.nlm.nih.gov/pubmed/?term=Shinohara%20M%5BAuthor%5D&cauthor=true&cauthor_uid=25726868). Cancer immunotherapy using novel tumor-associated antigenic peptides identified by genome-wide cDNA microarray analyses. *Cancer Sci* 2015; **106**: 505-511 [PMID: 25726868 DOI: 10.1111/cas.12650]

65 **Goldstein J**, Tran B, Ensor J, Gibbs P, Wong HL, Wong SF, Vilar E, Tie J, Broaddus R, Kopetz S, Desai J, Overman MJ. Multicenter retrospective analysis of metastatic colorectal cancer (CRC) with high-level microsatellite instability (MSI-H). *Ann Oncol* 2014; **25**: 1032-1038 [PMID: 24585723 DOI: 10.1093/annonc/mdu100]

66 [**Blankenstein T**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Blankenstein%20T%5BAuthor%5D&cauthor=true&cauthor_uid=25728991), [Leisegang M](http://www.ncbi.nlm.nih.gov/pubmed/?term=Leisegang%20M%5BAuthor%5D&cauthor=true&cauthor_uid=25728991), [Uckert W](http://www.ncbi.nlm.nih.gov/pubmed/?term=Uckert%20W%5BAuthor%5D&cauthor=true&cauthor_uid=25728991), [Schreiber H](http://www.ncbi.nlm.nih.gov/pubmed/?term=Schreiber%20H%5BAuthor%5D&cauthor=true&cauthor_uid=25728991). Targeting cancer-specific mutations by T cell receptor gene therapy. *Curr Opin Immunol* 2015; **33**: 112-119 [PMID: 25728991 DOI: 10.1016/j.coi.2015.02.005]

67 **Browning M**, Petronzelli F, Bicknell D, Krausa P, Rowan A, Tonks S, Murray N, Bodmer J, Bodmer W. Mechanisms of loss of HLA class I expression on colorectal tumor cells. *Tissue Antigens* 1996; **47**: 364-371 [PMID: 8795136]

68 **Kaklamanis L**, Townsend A, Doussis-Anagnostopoulou IA, Mortensen N, Harris AL, Gatter KC. Loss of major histocompatibility complex-encoded transporter associated with antigen presentation (TAP) in colorectal cancer. *Am J Pathol* 1994; **145**: 505-509 [PMID: 8080034]

69 **Rooney MS**, Shukla SA, Wu CJ, Getz G, Hacohen N. Molecular and genetic properties of tumors associated with local immune cytolytic activity. *Cell* 2015; **160**: 48-61 [PMID: 25594174 DOI: 10.1016/j.cell.2014.12.033]

70 **Hicklin DJ**, Marincola FM, Ferrone S. HLA class I antigen downregulation in human cancers: T-cell immunotherapy revives an old story. *Mol Med Today* 1999; **5**: 178-186 [PMID: 10203751]

71 **Nagorsen D**, Thiel E. HLA typing demands for peptide-based anti-cancer vaccine. *Cancer Immunol Immunother* 2008; **57**: 1903-1910 [PMID: 18317754 DOI: 10.1007/s00262-008-0493-6]

72 **Menon AG**, Morreau H, Tollenaar RA, Alphenaar E, Van Puijenbroek M, Putter H, Janssen-Van Rhijn CM, Van De Velde CJ, Fleuren GJ, Kuppen PJ. Down-regulation of HLA-A expression correlates with a better prognosis in colorectal cancer patients. *Lab Invest* 2002; **82**: 1725-1733 [PMID: 12480922]

73 **Tosolini M**, Kirilovsky A, Mlecnik B, Fredriksen T, Mauger S, Bindea G, Berger A, Bruneval P, Fridman WH, Pagès F, Galon J. Clinical impact of different classes of infiltrating T cytotoxic and helper cells (Th1, th2, treg, th17) in patients with colorectal cancer. *Cancer Res* 2011; **71**: 1263-1271 [PMID: 21303976 DOI: 10.1158/0008-5472.CAN-10-2907]

74 **Deschoolmeester V**, Baay M, Van Marck E, Weyler J, Vermeulen P, Lardon F, Vermorken JB. Tumor infiltrating lymphocytes: an intriguing player in the survival of colorectal cancer patients. *BMC Immunol* 2010; **11**: 19 [PMID: 20385003 DOI: 10.1186/1471-2172-11-19]

75 **Kobayashi M**, Kobayashi H, Pollard RB, Suzuki F. A pathogenic role of Th2 cells and their cytokine products on the pulmonary metastasis of murine B16 melanoma. *J Immunol* 1998; **160**: 5869-5873 [PMID: 9637498]

76 **O'Hara RJ**, Greenman J, MacDonald AW, Gaskell KM, Topping KP, Duthie GS, Kerin MJ, Lee PW, Monson JR. Advanced colorectal cancer is associated with impaired interleukin 12 and enhanced interleukin 10 production. *Clin Cancer Res* 1998; **4**: 1943-1948 [PMID: 9717823]

77 **Pellegrini P**, Berghella AM, Del Beato T, Cicia S, Adorno D, Casciani CU. Disregulation in TH1 and TH2 subsets of CD4+ T cells in peripheral blood of colorectal cancer patients and involvement in cancer establishment and progression. *Cancer Immunol Immunother* 1996; **42**: 1-8 [PMID: 8625361]

78 **Lahm H**, Schindel M, Frikart L, Cerottini JP, Yilmaz A, Givel JC, Fischer JR. Selective suppression of cytokine secretion in whole blood cell cultures of patients with colorectal cancer. *Br J Cancer* 1998; **78**: 1018-1023 [PMID: 9792144]

79 **Hawinkels LJ**, Paauwe M, Verspaget HW, Wiercinska E, van der Zon JM, van der Ploeg K, Koelink PJ, Lindeman JH, Mesker W, ten Dijke P, Sier CF. Interaction with colon cancer cells hyperactivates TGF-β signaling in cancer-associated fibroblasts. *Oncogene* 2014; **33**: 97-107 [PMID: 23208491 DOI: 10.1038/onc.2012.536]

80 **Massagué J**. The transforming growth factor-beta family. *Annu Rev Cell Biol* 1990; **6**: 597-641 [PMID: 2177343]

81 **Li MO**, Wan YY, Sanjabi S, Robertson AK, Flavell RA. Transforming growth factor-beta regulation of immune responses. *Annu Rev Immunol* 2006; **24**: 99-146 [PMID: 16551245 DOI: 10.1146/annurev.immunol.24.021605.090737]

82 **Friedman E**, Gold LI, Klimstra D, Zeng ZS, Winawer S, Cohen A. High levels of transforming growth factor beta 1 correlate with disease progression in human colon cancer. *Cancer Epidemiol Biomarkers Prev* 1995; **4**: 549-554 [PMID: 7549813]

83 **Narai S**, Watanabe M, Hasegawa H, Nishibori H, Endo T, Kubota T, Kitajima M. Significance of transforming growth factor beta1 as a new tumor marker for colorectal cancer. *Int J Cancer* 2002; **97**: 508-511 [PMID: 11802214 DOI: 10.1002/ijc.1631]

84 **Grady WM**, Myeroff LL, Swinler SE, Rajput A, Thiagalingam S, Lutterbaugh JD, Neumann A, Brattain MG, Chang J, Kim SJ, Kinzler KW, Vogelstein B, Willson JK, Markowitz S. Mutational inactivation of transforming growth factor beta receptor type II in microsatellite stable colon cancers. *Cancer Res* 1999; **59**: 320-324 [PMID: 9927040]

85 **Yan Z**, Deng X, Friedman E. Oncogenic Ki-ras confers a more aggressive colon cancer phenotype through modification of transforming growth factor-beta receptor III. *J Biol Chem* 2001; **276**: 1555-1563 [PMID: 11029459 DOI: 10.1074/jbc.M004553200]

86 **Gorelik L**, Flavell RA. Transforming growth factor-beta in T-cell biology. *Nat Rev Immunol* 2002; **2**: 46-53 [PMID: 11905837 DOI: 10.1038/nri704]

87 **Hsiao YW**, Liao KW, Hung SW, Chu RM. Tumor-infiltrating lymphocyte secretion of IL-6 antagonizes tumor-derived TGF-beta 1 and restores the lymphokine-activated killing activity. *J Immunol* 2004; **172**: 1508-1514 [PMID: 14734728]

88 **Whiteside TL**. Immune modulation of T-cell and NK (natural killer) cell activities by TEXs (tumour-derived exosomes). *Biochem Soc Trans* 2013; **41**: 245-251 [PMID: 23356291 DOI: 10.1042/BST20120265]

89 **Mapara MY**, Sykes M. Tolerance and cancer: mechanisms of tumor evasion and strategies for breaking tolerance. *J Clin Oncol* 2004; **22**: 1136-1151 [PMID: 15020616 DOI: 10.1200/JCO.2004.10.041]

90 **Asadullah K**, Sterry W, Volk HD. Interleukin-10 therapy--review of a new approach. *Pharmacol Rev* 2003; **55**: 241-269 [PMID: 12773629 DOI: 10.1124/pr.55.2.4]

91 **Galizia G**, Orditura M, Romano C, Lieto E, Castellano P, Pelosio L, Imperatore V, Catalano G, Pignatelli C, De Vita F. Prognostic significance of circulating IL-10 and IL-6 serum levels in colon cancer patients undergoing surgery. *Clin Immunol* 2002; **102**: 169-178 [PMID: 11846459 DOI: 10.1006/clim.2001.5163]

92 **Ling L**, Zhao P, Yan G, Chen M, Zhang T, Wang L, Jiang Y. The frequency of Th17 and Th22 cells in patients with colorectal cancer at pre-operation and post-operation. *Immunol Invest* 2015; **44**: 56-69 [PMID: 25026244 DOI: 10.3109/08820139.2014.936445]

93 **Nelson BH**. CD20+ B cells: the other tumor-infiltrating lymphocytes. *J Immunol* 2010; **185**: 4977-4982 [PMID: 20962266 DOI: 10.4049/jimmunol.1001323]

94 [**Linnebacher M**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Linnebacher%20M%5BAuthor%5D&cauthor=true&cauthor_uid=23170274), [Maletzki C](http://www.ncbi.nlm.nih.gov/pubmed/?term=Maletzki%20C%5BAuthor%5D&cauthor=true&cauthor_uid=23170274). Tumor-infiltrating B cells: The ignored players in tumor immunology. *Oncoimmunology* 2012; **1**: 1186-1188 [PMID: 23170274 DOI: 10.4161/onci.20641]

95 **Palucka K**, Ueno H, Roberts L, Fay J, Banchereau J. Dendritic cells: are they clinically relevant? *Cancer J* 2010; **16**: 318-324 [PMID: 20693842 DOI: 10.1097/PPO.0b013e3181eaca83]

96 **Fong L**, Engleman EG. Dendritic cells in cancer immunotherapy. *Annu Rev Immunol* 2000; **18**: 245-273 [PMID: 10837059 DOI: 10.1146/annurev.immunol.18.1.245]

97 **Suzuki A**, Masuda A, Nagata H, Kameoka S, Kikawada Y, Yamakawa M, Kasajima T. Mature dendritic cells make clusters with T cells in the invasive margin of colorectal carcinoma. *J Pathol* 2002; **196**: 37-43 [PMID: 11748640 DOI: 10.1002/path.1018]

98 **Dadabayev AR**, Sandel MH, Menon AG, Morreau H, Melief CJ, Offringa R, van der Burg SH, Janssen-van Rhijn C, Ensink NG, Tollenaar RA, van de Velde CJ, Kuppen PJ. Dendritic cells in colorectal cancer correlate with other tumor-infiltrating immune cells. *Cancer Immunol Immunother* 2004; **53**: 978-986 [PMID: 15197496 DOI: 10.1007/s00262-004-0548-2]

99 **Banchereau J**, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, Palucka K. Immunobiology of dendritic cells. *Annu Rev Immunol* 2000; **18**: 767-811 [PMID: 10837075 DOI: 10.1146/annurev.immunol.18.1.767]

100 **Schwaab T**, Weiss JE, Schned AR, Barth RJ. Dendritic cell infiltration in colon cancer. *J Immunother* 2001; **24**: 130-137 [PMID: 11265770]

101 **Bauer K**, Michel S, Reuschenbach M, Nelius N, von Knebel Doeberitz M, Kloor M. Dendritic cell and macrophage infiltration in microsatellite-unstable and microsatellite-stable colorectal cancer. *Fam Cancer* 2011; **10**: 557-565 [PMID: 21598004 DOI: 10.1007/s10689-011-9449-7]

102 **Steinman RM**, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. *Annu Rev Immunol* 2003; **21**: 685-711 [PMID: 12615891 DOI: 10.1146/annurev.immunol.21.120601.141040]

103 **Whiteside TL**. Regulatory T cell subsets in human cancer: are they regulating for or against tumor progression? *Cancer Immunol Immunother* 2014; **63**: 67-72 [PMID: 24213679 DOI: 10.1007/s00262-013-1490-y]

104 **Michielsen AJ**, Hogan AE, Marry J, Tosetto M, Cox F, Hyland JM, Sheahan KD, O'Donoghue DP, Mulcahy HE, Ryan EJ, O'Sullivan JN. Tumour tissue microenvironment can inhibit dendritic cell maturation in colorectal cancer. *PLoS One* 2011; **6**: e27944 [PMID: 22125641 DOI: 10.1371/journal.pone.0027944]

105 **Koda K**, Saito N, Takiguchi N, Oda K, Nunomura M, Nakajima N. Preoperative natural killer cell activity: correlation with distant metastases in curatively research colorectal carcinomas. *Int Surg* 1997; **82**: 190-193 [PMID: 9331851]

106 [**Sconocchia G**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Sconocchia%20G%5BAuthor%5D&cauthor=true&cauthor_uid=25610741), [Eppenberger S](http://www.ncbi.nlm.nih.gov/pubmed/?term=Eppenberger%20S%5BAuthor%5D&cauthor=true&cauthor_uid=25610741), [Spagnoli GC](http://www.ncbi.nlm.nih.gov/pubmed/?term=Spagnoli%20GC%5BAuthor%5D&cauthor=true&cauthor_uid=25610741), [Tornillo L](http://www.ncbi.nlm.nih.gov/pubmed/?term=Tornillo%20L%5BAuthor%5D&cauthor=true&cauthor_uid=25610741), [Droeser R](http://www.ncbi.nlm.nih.gov/pubmed/?term=Droeser%20R%5BAuthor%5D&cauthor=true&cauthor_uid=25610741), [Caratelli S](http://www.ncbi.nlm.nih.gov/pubmed/?term=Caratelli%20S%5BAuthor%5D&cauthor=true&cauthor_uid=25610741), [Ferrelli F](http://www.ncbi.nlm.nih.gov/pubmed/?term=Ferrelli%20F%5BAuthor%5D&cauthor=true&cauthor_uid=25610741), [Coppola A](http://www.ncbi.nlm.nih.gov/pubmed/?term=Coppola%20A%5BAuthor%5D&cauthor=true&cauthor_uid=25610741), [Arriga R](http://www.ncbi.nlm.nih.gov/pubmed/?term=Arriga%20R%5BAuthor%5D&cauthor=true&cauthor_uid=25610741), [Lauro D](http://www.ncbi.nlm.nih.gov/pubmed/?term=Lauro%20D%5BAuthor%5D&cauthor=true&cauthor_uid=25610741), [Iezzi G](http://www.ncbi.nlm.nih.gov/pubmed/?term=Iezzi%20G%5BAuthor%5D&cauthor=true&cauthor_uid=25610741), [Terracciano L](http://www.ncbi.nlm.nih.gov/pubmed/?term=Terracciano%20L%5BAuthor%5D&cauthor=true&cauthor_uid=25610741), [Ferrone S](http://www.ncbi.nlm.nih.gov/pubmed/?term=Ferrone%20S%5BAuthor%5D&cauthor=true&cauthor_uid=25610741). NK cells and T cells cooperate during the clinical course of colorectal cancer. *Oncoimmunology* 2014; **3**: e952197 [PMID: 25610741 DOI: 10.4161/21624011.2014.952197]

107 **Remark R**, Alifano M, Cremer I, Lupo A, Dieu-Nosjean MC, Riquet M, Crozet L, Ouakrim H, Goc J, Cazes A, Fléjou JF, Gibault L, Verkarre V, Régnard JF, Pagès ON, Oudard S, Mlecnik B, Sautès-Fridman C, Fridman WH, Damotte D. Characteristics and clinical impacts of the immune environments in colorectal and renal cell carcinoma lung metastases: influence of tumor origin. *Clin Cancer Res* 2013; **19**: 4079-4091 [PMID: 23785047 DOI: 10.1158/1078-0432.CCR-12-3847]

108 **Peng YP**, Zhu Y, Zhang JJ, Xu ZK, Qian ZY, Dai CC, Jiang KR, Wu JL, Gao WT, Li Q, Du Q, Miao Y. Comprehensive analysis of the percentage of surface receptors and cytotoxic granules positive natural killer cells in patients with pancreatic cancer, gastric cancer, and colorectal cancer. *J Transl Med* 2013; **11**: 262 [PMID: 24138752 DOI: 10.1186/1479-5876-11-262]

109 **Biroccio A**, Cherfils-Vicini J, Augereau A, Pinte S, Bauwens S, Ye J, Simonet T, Horard B, Jamet K, Cervera L, Mendez-Bermudez A, Poncet D, Grataroli R, de Rodenbeeke CT, Salvati E, Rizzo A, Zizza P, Ricoul M, Cognet C, Kuilman T, Duret H, Lépinasse F, Marvel J, Verhoeyen E, Cosset FL, Peeper D, Smyth MJ, Londoño-Vallejo A, Sabatier L, Picco V, Pages G, Scoazec JY, Stoppacciaro A, Leonetti C, Vivier E, Gilson E. TRF2 inhibits a cell-extrinsic pathway through which natural killer cells eliminate cancer cells. *Nat Cell Biol* 2013; **15**: 818-828 [PMID: 23792691 DOI: 10.1038/ncb2774]

110 [**Subramani B**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Subramani%20B%5BAuthor%5D&cauthor=true&cauthor_uid=24944796), [Pullai CR](http://www.ncbi.nlm.nih.gov/pubmed/?term=Pullai%20CR%5BAuthor%5D&cauthor=true&cauthor_uid=24944796), [Krishnan K](http://www.ncbi.nlm.nih.gov/pubmed/?term=Krishnan%20K%5BAuthor%5D&cauthor=true&cauthor_uid=24944796), [Sugadan SD](http://www.ncbi.nlm.nih.gov/pubmed/?term=Sugadan%20SD%5BAuthor%5D&cauthor=true&cauthor_uid=24944796), [Deng X](http://www.ncbi.nlm.nih.gov/pubmed/?term=Deng%20X%5BAuthor%5D&cauthor=true&cauthor_uid=24944796), [Hiroshi T](http://www.ncbi.nlm.nih.gov/pubmed/?term=Hiroshi%20T%5BAuthor%5D&cauthor=true&cauthor_uid=24944796), [Ratnavelu K](http://www.ncbi.nlm.nih.gov/pubmed/?term=Ratnavelu%20K%5BAuthor%5D&cauthor=true&cauthor_uid=24944796). Efficacy of ex vivo activated and expanded natural killer cells and T lymphocytes for colorectal cancer patients. *Biomed Rep* 2014; **2**: 505-508 [PMID: 24944796 DOI: 10.3892/br.2014.264]

111 **Rocca YS**, Roberti MP, Arriaga JM, Amat M, Bruno L, Pampena MB, Huertas E, Loria FS, Pairola A, Bianchini M, Mordoh J, Levy EM. Altered phenotype in peripheral blood and tumor-associated NK cells from colorectal cancer patients. *Innate Immun* 2013; **19**: 76-85 [PMID: 22781631 DOI: 10.1177/1753425912453187]

112 **Coca S**, Perez-Piqueras J, Martinez D, Colmenarejo A, Saez MA, Vallejo C, Martos JA, Moreno M. The prognostic significance of intratumoral natural killer cells in patients with colorectal carcinoma. *Cancer* 1997; **79**: 2320-2328 [PMID: 9191519]

113 **Tallerico R**, Todaro M, Di Franco S, Maccalli C, Garofalo C, Sottile R, Palmieri C, Tirinato L, Pangigadde PN, La Rocca R, Mandelboim O, Stassi G, Di Fabrizio E, Parmiani G, Moretta A, Dieli F, Kärre K, Carbone E. Human NK cells selective targeting of colon cancer-initiating cells: a role for natural cytotoxicity receptors and MHC class I molecules. *J Immunol* 2013; **190**: 2381-2390 [PMID: 23345327 DOI: 10.4049/jimmunol.1201542]

114 **Josefowicz SZ**, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. *Annu Rev Immunol* 2012; **30**: 531-564 [PMID: 22224781 DOI: 10.1146/annurev.immunol.25.022106.141623]

115 **deLeeuw RJ**, Kost SE, Kakal JA, Nelson BH. The prognostic value of FoxP3+ tumor-infiltrating lymphocytes in cancer: a critical review of the literature. *Clin Cancer Res* 2012; **18**: 3022-3029 [PMID: 22510350 DOI: 10.1158/1078-0432.CCR-11-3216]

116 **Ladoire S**, Martin F, Ghiringhelli F. Prognostic role of FOXP3+ regulatory T cells infiltrating human carcinomas: the paradox of colorectal cancer. *Cancer Immunol Immunother* 2011; **60**: 909-918 [PMID: 21644034 DOI: 10.1007/s00262-011-1046-y]

117 **Kryczek I**, Liu R, Wang G, Wu K, Shu X, Szeliga W, Vatan L, Finlayson E, Huang E, Simeone D, Redman B, Welling TH, Chang A, Zou W. FOXP3 defines regulatory T cells in human tumor and autoimmune disease. *Cancer Res* 2009; **69**: 3995-4000 [PMID: 19383912 DOI: 10.1158/0008-5472.CAN-08-3804]

118 **Chaput N**, Louafi S, Bardier A, Charlotte F, Vaillant JC, Ménégaux F, Rosenzwajg M, Lemoine F, Klatzmann D, Taieb J. Identification of CD8+CD25+Foxp3+ suppressive T cells in colorectal cancer tissue. *Gut* 2009; **58**: 520-529 [PMID: 19022917 DOI: 10.1136/gut.2008.158824]

119 **Erdman SE**, Poutahidis T, Tomczak M, Rogers AB, Cormier K, Plank B, Horwitz BH, Fox JG. CD4+ CD25+ regulatory T lymphocytes inhibit microbially induced colon cancer in Rag2-deficient mice. *Am J Pathol* 2003; **162**: 691-702 [PMID: 12547727 DOI: 10.1016/S0002-9440(10)63863-1]

120 **Delgoffe GM**, Woo SR, Turnis ME, Gravano DM, Guy C, Overacre AE, Bettini ML, Vogel P, Finkelstein D, Bonnevier J, Workman CJ, Vignali DA. Stability and function of regulatory T cells is maintained by a neuropilin-1-semaphorin-4a axis. *Nature* 2013; **501**: 252-256 [PMID: 23913274 DOI: 10.1038/nature12428]

121 **Tel J**, Smits EL, Anguille S, Joshi RN, Figdor CG, de Vries IJ. Human plasmacytoid dendritic cells are equipped with antigen-presenting and tumoricidal capacities. *Blood* 2012; **120**: 3936-3944 [PMID: 22966165 DOI: 10.1182/blood-2012-06-435941]

122 **Rabinovich GA**, Gabrilovich D, Sotomayor EM. Immunosuppressive strategies that are mediated by tumor cells. *Annu Rev Immunol* 2007; **25**: 267-296 [PMID: 17134371 DOI: 10.1146/annurev.immunol.25.022106.141609]

123 **Izhak L**, Ambrosino E, Kato S, Parish ST, O'Konek JJ, Weber H, Xia Z, Venzon D, Berzofsky JA, Terabe M. Delicate balance among three types of T cells in concurrent regulation of tumor immunity. *Cancer Res* 2013; **73**: 1514-1523 [PMID: 23319803 DOI: 10.1158/0008-5472.CAN-12-2567]

124 **Cordero OJ**, Salgado FJ, Nogueira M. On the origin of serum CD26 and its altered concentration in cancer patients. *Cancer Immunol Immunother* 2009; **58**: 1723-1747 [PMID: 19557413 DOI: 10.1007/s00262-009-0728-1]

125 **Cordero OJ**, Imbernon M, Chiara LD, Martinez-Zorzano VS, Ayude D, de la Cadena MP, Rodriguez-Berrocal FJ. Potential of soluble CD26 as a serum marker for colorectal cancer detection. *World J Clin Oncol* 2011; **2**: 245-261 [PMID: 21773075 DOI: 10.5306/wjco.v2.i6.245]

126 **Cherciu I**, Bărbălan A, Pirici D, Mărgăritescu C, Săftoiu A. Stem cells, colorectal cancer and cancer stem cell markers correlations. *Curr Health Sci J* 2014; **40**: 153-161 [PMID: 25729599 DOI: 10.12865/CHSJ.40.03.01]

127 **Zhou J**, Belov L, Chapuis P, Chan C, Armstrong N, Kaufman KL, Solomon MJ, Clarke SJ, Christopherson RI. Surface profiles of live colorectal cancer cells and tumor infiltrating lymphocytes from surgical samples correspond to prognostic categories. *J Immunol Methods* 2015; **416**: 59-68 [PMID: 25445327 DOI: 10.1016/j.jim.2014.11.001]

**P- Reviewer:** Jin B, Linnebacher M, Riccardi C **S- Editor:** Gong XM

**L- Editor:** **E- Editor:**