What roles do colon stem cells and gap junctions play in the left and right location of origin of colorectal cancers?

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Abstract This “Commentary” examines an important clinical observation that right-sided colorectal cancers appear less treatable than the left-sided cancers. The concepts of (a) the “initiation/promotion/progression” process, (b) the stem cell hypothesis, (c) the role gap junctional intercellular communication, (d) cancer cells lacking GJIC either because of the non-expression of connexin genes or of non-functional gap junction proteins, and (e) the role of the microbiome in promoting initiated colon stem cells to divide symmetrically or asymmetrically are examined to find an explanation. It has been speculated that “embryonic-like” lesions in the ascending colon are initiated stem cells, promoted via symmetrical cell division, while the polyp-type lesions in the descending colon are initiated stem cells stimulated to divide asymmetrically. To test this hypothesis, experiments could be designed to examine if right-sided lesions might express Oct4A and ABCG2 genes but not any connexin genes, whereas the left-sided lesions might express a connexin gene, but not Oct4A or the ABCG2 genes. Treatment of the right sided lesions might include transcriptional regulators, whereas the left-sided lesions would need to restore the posttranslational status of the connexin proteins.

Keywords Colorectal cancers · Adult stem cells · Gap junction intercellular communication · Colon stem cells · Cancer stem cells · Connexin genes · Microbiome

Introduction

With the important observation that the location of the origin of colorectal cancers seems to determine the lethality of the tumors (Loupakis et al. 2015a, b), the significance of this observation is telling us to look for some fundamental biological underpinning that might serve to provide guides to either or both prevention and treatment of colon cancers. Metastatic colorectal cancers (CRC) can be defined based on the location of the primary tumour. Historically, publications have defined CRCs within three compartments of the gut: distal colon, proximal colon and rectum (Bufill 1990; Gervaz et al. 2004; Iacopetta 2002). Right-sided colon carcinomas (RCC) are located within the midgut, which encompasses the proximal two-thirds of the transverse colon, ascending colon and caecum. Left-sided colon carcinomas (LCC) lie within the hindgut, which includes the known as the descending colon, sigmoid colon and rectum. Patients with RCC and LCC differ in their microbiome, clinical characteristics, molecular profiling, clinical outcome and response to treatment. However, embryology cannot explain these differences since the midgut and hindgut are derived from the same origin, the endoderm, and the rectum derives from the cloacal membrane (Hill 2016; Sadler 2014). Microbiome differs significantly between the right- and left-sided colon which may explain the gradual changes in MSI and CIMP types of CRC along the length of the gut (Yamauchi et al. 2012; Gagniere et al. 2016). Regional differences in the physiological functioning of the right & left regions of the colon might expose these two types of initiated stem cells could affect either the anti-mitogenic factors (niche

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extra-cellular matrices and soluble factors, low oxygen micro-environment) for the non-expressing connexin stem cells or the contact-inhibiting factors (pollutants; dietary components; drugs, hormones, growth factors, cytokines, microbial toxins, etc.) for the connexin-expressing, but non GJIC-functioning initiated stem cells.”

LCC are more chromosomally instable and RCC have a higher frequency of MSI-high phenotypes, and KRAS and BRAF mutations (Venook et al. 2016; Heinemann et al. 2014), which may account for the poorer prognosis of these patients. Location of the primary tumor is a known prognostic factor for patients with CRC (Loupakis et al. 2015; Nitsche et al. 2016; Price et al. 2015; Sinicrope et al. 2015; Venook et al. 2016; Wong et al. 2016). RCC are associated with a poorer prognosis than LCC but may be also predictive for anti-EGFR and anti-angiogenic therapies. (Loupakis et al. 2015a, b; Venook et al.; 2016; Wong et al. 2016; Houts et al. 2016; Lenz et al. 2016)

As with all scientific and medical problems, we have a choice to tackle the problem either with an empirical or a hypothesis-testing experimental approach. Given today's propensity to use powerful and sophisticated technologies to solve medical problems, much of current cancer research (basic; chemo-preventive; chemo-therapeutic; translational, etc.) hopes to generate massive amounts of data, from which various uses of different algorithms of bioinformatics will lead to identification of critical patterns for “personalized” medicine. Yet, the alternative approach to use biologically-based hypothesis-testing seems to depend on the apparent lack of universally-accepted mechanisms to explain the complex multi-stage, multi-mechanism origin of all cancers. The seemingly complex interactions of genetic, developmental, gender, physical/chemical/microbiological agent interactions, doses/concentrations, chemical mixture interactions affecting the redox state of cells, psychological, behavioral, social and cultural factors make for simple hypothesis-testing very difficult. Any cancer researcher knows that to submit a research proposal with a hypothesis-testing strategy will meet with a great chance of a critical review, challenging the hypothesis used to propose the experimental testing, compared to a proposal with no hypothesis being proposed but with the highlighting of the state of the art technologies to generate tons of data.

It is within this broad background that there exists a rationale for a hypothesis to explain this observation that the location of the polyp-type, partially differentiated colorectal cancers is different from the flat, undifferentiated-type colorectal tumors that, somehow, makes one type more treatable than the other. The rationale for the hypothesis to be presented is independent on the reality that the former type is easily detected during colonoscopy, whereas the flat-type is more easily missed. The hypothesis to be proposed here is founded on an old, but solid, observation that Loewenstein and Kanno made decades ago (1966), namely, that all cancer cells, unlike

normal cells, lack gap junctional intercellular communication. Before expanding on his observation, it should be noted that his papers are rarely cited by the most cited cancer researchers. In addition, while the role of gap junctions in the carcinogenic process is clearly complex, and the link between “cancer cells” and the lack of functional gap junctions in cancer cells, itself, is complex (Aasen et al. 2016; Leithe et al. 2006; Saez 2016; Jiang and Penuela 2016; Trosko 2016a, b) such as the observation of “selective” lack of gap junctional intercellular communication (Yamasaki et al. 1987), the assumption being made here is that GJIC is a necessary, if not sufficient, requirement for a cancer cell to be immortal, have uncontrolled growth, cannot terminally differentiate and does not apoptose correctly.

In order to characterize the origin of cancer cells, two additional hypotheses have driven cancer research, off and on, for many years, namely that: (a) cancers originate from adult-organ-specific stem cells (Markert 1968; Pierce 1974; Fialkow 1976; Potter 1978; Till 1982; Trosko and Tai 2006) or (b) from the “de-differentiation” or “re-programming” of somatic differentiated cells (Sell 1993). Only with the discovery of human embryonic stem cells (Bongso et al. 1994; Thomson et al. 1998; Shambloott et al. 1998), induced-pluripotent stem cells (Takahashi and Yamanaka 2006), organ-specific adult human stem cells (Tai et al. 2005), as well as the re-introduction of the idea of “cancer-initiating” or “cancer stem cells” (Trosko 2014) has it been possible to start to characterize these normal stem cells and cancer stem cells. While it can be said, up front, that there is no universal acceptance of which of these two hypotheses might explain what is the “target cell” for cancers, there is some strong evidence that the adult organ-specific stem cell is the “target” cell for the conversion of this adult stem cell to be initiated (Tai et al. 2005; Bonnet and Dick 1997; Barker et al. 2009; Wang et al. 2009; Sanchez et al. 2016). The interpretation of an organ-specific adult stem cell being “initiated” by some agent that can influence carcinogenesis is that this initiated adult stem cell will be unable to terminally differentiate and remain in its normal “undifferentiated” state [or promoted] (Trosko and Kang 2012), to accrue all those other mutagenic and epigenetic changes to acquire the “hallmarks” of cancer (Hanahan and Weinberg 2000; Hanahan and Weinberg 2011).

Yet, this alone will not explain the original differences in the left and right origin of two different kinds of cancer. Another series of observations, usually dismissed in current cancer research papers and research proposals, was built on Loewenstein's original characterization of the lack of gap junctional intercellular communication (GJIC) in cancer cells. That observation came from historic observations on the ability of some chemicals and physical conditions that could cause a single “initiated” cell to be selectively expanded by hyperplastic growth and a loss of apoptosis (Berenblum 1954; Yamagiwa and Ichikawa 1977; Trosko 2001). This

process, seen in skin, liver, breast, and bladder tissues in vivo, was termed, *tumor promotion* (Weinstein et al. 1984; Pitot and Dragon 1991). What is interesting about these tumor-promoting chemicals is that they were not mutagenic, but acted by “epigenetic” mechanisms (Trosko et al. 1990; Trosko and Ruch 1998; Trosko et al. 1998; Upham and Trosko 2009). In brief, the powerful mouse skin tumor promoter (phorbol ester or TPA), as shown to inhibit, reversibly, GJIC (Yotti et al. 1979). Later, more non-mutagenic chemicals, e.g., DDT, phthalates, phenobarbital, etc., could inhibit GJIC, reversibly, by triggering redox changes in cells and trigger various intracellular signals to alter GJIC (Upham and Trosko 2009; Trosko and Chang 1988). Species specificity and threshold levels for these tumor promoting chemicals were discovered (Pitot and Dragon 1991; Klaunig and Ruch 1987).

Later, using the logic that there exist chemicals that could reversibly inhibit GJIC to be tumor promoters, there might be chemicals that could do exactly the opposite to GJIC. This led to predict, successfully, that both natural and synthetic chemicals do exist, which could either prevent GJIC-inhibiting tumor promoters or that could enhance, transcriptionally, enhanced expression of connexin genes. (Trosko and Ruch 2002; Leone et al. 2012).

In addition, various oncogenes, such as src, ras, and neu, could stably inhibit gap junctions (Trosko and Ruch 1998). This observation starts to provide some insight as to why there might be **two types of cancer cells** that do not have functional GJIC intercellular communication (Trosko 2003). To understand the basis of this statement, another observation, rarely mentioned, is that stem cells (embryonic, induced pluripotent stem cells, adult organ-specific stem cells) do not express their gap junction or connexin genes [of which there are twenty in this highly evolutionarily-conserved family (Cruciani and Mikalsen 2006)]. While there have been contradictory studies suggesting the expression of connexin genes or the functionality of GJIC in various types of stem cells, due to different methodologies used, the fact that embryonic-, induced pluripotent- and organ-specific- stem cells are propagated on feeder layer cells, suggests that there was no GJIC between the stem cells and the feeder layer cells. One example is seen in Figs. 5 and 6 (Chang et al. 1987) and Fig. 3 (Kao et al. 1995). This observation that stem cells do not express their connexin genes or have functional GJIC is because of the evolutionary role of gap junctions to help maintain homeostatic control of cell proliferation, cell differentiation and apoptosis (Trosko 2016a, b). While the evolutionary appearance of this family of connexin or gap junction genes, as well as the germinal and somatic stem cells (Crosnier et al. 2006; Mentink and Tsiantis 2015; Weissman 2015; Horn et al. 2015), has yet to be explained, the appearance of multicellularity and gap junctions and stem cells seemed to depend on each other (Trosko 2016a, b).

With the accepted definition of a stem cell as a cell having the ability to divide either symmetrically to maintain self-renewal or by asymmetrical division to produce one daughter that maintains self-renewal and another daughter that can terminally differentiate, it seems that, while there are obvious genes needed to control which division process to initiate, critical endogenous and exogenous factors must trigger the symmetrical or asymmetrical division process during development and subsequent state of health during adolescent, mature and geriatric stages of life (Lane et al. 2014). With the stem cell seemingly having metabolic characteristics of anaerobic single cell organisms, namely, metabolism of glucose via glycolysis, having few mitochondria (Neste et al. 2007; Armstrong et al. 2010; Chen et al. 2008; Prigione et al. 2010), and aversion to high levels of oxygen with their protective niches (Csete 2005; Pervaiz et al. 2009; Mohyeldin et al. 2010; Brigelius-Flohé and Flohé 2011; Atena et al. 2014), the stem cell's phenotype mimics that of the single cell organism (Trosko 2014). In addition, given the old observation that cancer cells exhibit the Warburg metabolism (Warburg 1956) and the more recent identification of the “cancer initiating” or “cancer stem cells” (Al Hajj et al. 2003; Cozzio et al. 2003), as well as evidence that the organ-specific adult stem cell as being the origin of these “cancer stem” cells (Tai et al. 2005; Hemmati et al. 2003; Hope et al. 2003; Singh et al. 2003; Galli et al. 2004; Kondo et al. 2004; Yuan et al. 2004; De Jong et al. 2005; Locke et al. 2005; Ponti et al. 2005; Krivtsov et al. 2006; Li and Yurchenco 2006; Gu et al. 2007; O'Brien et al. 2007; Ricci-Vitiani et al. 2007; Wu et al. 2007; Schatton et al. 2008), several observations bring the stem cell hypothesis and “de-differentiation” or “reprogramming” hypothesis of cancer for further examination.

One of the prevailing paradigms in the cancer field is that the first step of the “initiation”, “promotion” and “progression” process of carcinogenesis is the “immortalization” of a normal “mortal cell” (Land et al. 1983). In effect, it is generally assumed that the normal, mortal cell, which was “initiated” or “immortalized”, was a somatic differentiated cell. Even more recent evidence of the Nobel Prize work by S. Yamanaka, that one could “re-program” a normal differentiated cell by the genetic insertion of a few “embryonic genes” (Takahashi and Yamanaka 2006), seemed to support the “de-differentiation” or “reprogramming” hypothesis of the cancer process. However, an alternative hypothesis has been proposed (Trosko 2006, 2008a, b, 2009, 2014).

In brief, if one assumes that a stem cell is fundamentally “immortal” until it is induced to terminally differentiate or to become “mortal”, then there are important facts, for which there must be some accounting. First, all organs of the human body are known to have organ-specific adult stem cells (e.g., skin, liver, intestine, eye, brain, blood, pancreas, kidney, ovary, testes, bone, lung, etc.). Second, in all early primary in vitro cultures of tissues, there are a few adult organ-specific adult

stem cells, which, when grown under typical in vitro conditions (e.g., on plastic; high oxygen levels, etc.), are lost after a few passages. That is why primary cultures usually die out after a finite number of passages [Hayflick phenomenon] (Hayflick 1965). Third, when one places the Yamanaka-type “induced pluripotent stem cells” back into an adult host animal, they form teratomas. ***If, in fact, cancers are induced in vivo in human beings by the “re-programming” process of “immortalizing” a differentiated somatic cell, then teratomas, not sarcomas or carcinomas, would be formed.*** Last, there is strong evidence that adult stem cells are the target cells for initiating the cancer (Tai et al. 2005; Bonnet and Dick 1997; Barker et al. 2009; Wang et al. 2009; Sanchez et al. 2016).

If the organ-specific adult stem cell, which, by definition, has an unlimited life span and which, under normal conditions, mutations could be formed by “error-prone DNA repair” (Cleaver and Trosko 1970; Maher and McCormick 1976; Cleaver 1978; Glover et al. 1979; Brash et al. 1991) or “error-prone- DNA Replication (Warren et al. 1981). If those mutations occurred in a stem cell’s genes to regulate asymmetrical cell division, but does not affect symmetrical cell division, then that stem cell would be able to expand but not terminally differentiate (Kang and Trosko 2011). ***In effect, the initiation process does not induce “immortalization” of a “mortal” somatic differentiated cell, but it blocks “mortalization” or terminal differentiation of an “immortal” adult stem cell.***

At this point, the observation of “immortalizing viruses”, such as SV40 and human papilloma viruses, should be brought into this analysis (Aasen et al. 2016). Historically, in the many attempts to obtain transformed cells in vitro by exposing normal primary cultured cells to various radiations or carcinogenic chemicals, nothing but failures were reported (DiPaolo 1983; Kuroki and Huh 1993; Rhim 1993). Only with the infection or transfection of the immortalizing viruses or some of their genes in primary fibroblastic or epithelial cells, could one isolate a few “immortalized” cells. Hence, the original logical designation of the terms, “immortalizing viruses” occurred. However, another interpretation of these observations can, again, be made. In those primary cultures, there exist a few adult organ-specific stem cells. If, these viruses or their “immortalizing” genes, infected or transfected, all the cells (differentiated and the few adult stem cells), only the stem cells survived the subsequent passages. The infected differentiated cells died during “crisis period” via the Hayflick phenomenon. However, if the SV40 and HPV E-6, E-7 genes rendered the p53 and RB gene products unable to bring about differentiation of the stem cells (Ahuja et al. 2005), these cells remain “immortal”, undifferentiated, since they were not “re-programmed” to convert their differentiated state or state of “mortality” to that of the “immortalized” phenotype. This is a radical change of the paradigm to explain the first step of carcinogenesis.

If this explanation is correct, how might this be of help to either prevent or treat colorectal cancers, based on the observation that right-sided tumors have a worse prognosis than those of the left sided colorectal tumors? An explanation can be offered. It is based on the assumption that, if all tumors contain cells that cannot perform GJIC or performs “selective” GJIC (Yamasaki et al. 1987) , which is needed for “contact inhibition” or growth control (Eagle 1965), needed for differentiation (Yamasaki and Naus 1996) and for apoptosis (Wilson et al. 2000), then one must note that there exist two kinds of cancer cells that do not have functional GJIC (Trosko 2007). There are those cancer cells, which started from a stem cell that was “initiated” before the connexin genes were transcriptionally expressed. These cells are “frozen” in their “immortalized” and undifferentiated state. While not being neoplastically- transformed at this “initiated” stage, they can still be mitogenically- repressed by insoluble or soluble anti-mitogenic growth factors, such as niche- extracellular matrix molecules or negative soluble growth- regulators that bind to their receptors and signal transducers that control mitogenesis.

Given that prevention of initiation of carcinogenesis can be reduced, but not to zero levels, due to the normal production of mutations by “errors of replication” caused by normal stem cell replication, the **promotion phase of carcinogenesis** is the “rate-limiting” step of carcinogenesis, as it takes place of decades in adults (Trosko 2006). Therefore, in both the right and left regions of the colon, promotion is the process that would cause the initiated stem cell expansion. The promotion process requires an endogenous (growth factor, hormone, cytokine) or exogenous (pollutant, medication, microbial toxin, dietary chemical, etc.) factors to release the initiated stem cell from anti-mitogenic agents, as well to block apoptotic factors, in order to expand this pre-malignant population. In addition, promoters must exceed “threshold” levels in order to block gap junctional intercellular communication, while at the same time, work in the absence of “anti-promoters” (Leone et al. 2012). ***Only after stimulation by “promoters” that release these “initiated”, undifferentiated stem cells from anti-mitotic restraint, could these initiated stem cells expand to form flat, undifferentiated or “embryonic-like” lesions.***

On the other hand, if an early stem cell, which has its connexin genes transcriptionally expressed, but has either (a) an expressed mutated oncogene, such as neu, src, or ras, these oncogene products can, posttranslationally, modify the connexin proteins to render them non-functional (Trosko and Ruch 2002) or (b) exposed chronically to promoters that can inhibit gap junctions, then these cells can be clonally amplified or promoted to form polyp-type lesions. These cells are also phenotypically like the initiated stem cell, in that they both are unable to communicate intercellular signals for growth control.

So, what might be the promoters of these two types of “initiated” stem cells? There are many agents that can inhibit

mitogenesis of (a) an initiated stem cell without expressed connexins or gap junctions or (b) a contact-inhibited, gap junctional intercellular communication-expressing, partially differentiated stem cells. This is a major barrier in screening for a “specific” promoter for colon cancers. So many factors can cause “promotion” of either type of “initiated” colon cancer stem cell. This is seen in a major observation that, depending on the stage of development when initiation occurred (pre- and post-weaning), the tumor promoter, phenobarbital, affected two types of lesions in the rat (Lee 2000)

To recapitulate the logical chain of thought, human intestinal tissue contains adult stem cells (Barker et al. 2007; Potten et al. 2009; Kemper et al. 2012; Sato and Clevers 2013). If the adult stem cell is capable of either symmetrical or asymmetrical cell division, depending on endogenous or exogenous signals it receives (Barker 2014), then either expansion of the stem cell population or of its progenitor/differentiated lineages will follow. Also, if one assumes that these intestinal stem cells are the “target” cells for colon cancers (Huels and Sansom 2015), the first step of the carcinogenic process is to block its ability to divide asymmetrically under normal conditions to be “initiated” (Trosko 2016a, b). However, if that stem cell is “initiated” in the stem cell state, and “promoted” by agents (endogenous or exogenous) that favors symmetrical cell division, these initiated colon stem cell cells will form, undifferentiated-type or “flat” lesions. On the other hand, if the initiated colon stem cell is promoted by endogenous or exogenous factors that can force some “partial differentiation (“oncogeny as partially block ontogeny”) (Potter 1978), then lesions will form polyps.

One has to note that normal growth modulators of cell proliferation, such as growth factors (Ruch et al. 2001), cytokines and chemokines (Martin and Prince 1982; Brosnan et al. 2001), hormones (Puri and Garfield 1982), as well as dietary factors and drugs and pollutants, and products of food preparation (Dougal et al. 2012), can alter GJIC. Therefore, the question arises, “*Why are the lesions arising from the right-sided adult colon initiated stem cells giving rise to “flat lesions, while those adult initiated stem cells promoted to form the polyp –type partially differentiated lesion”?* Could it be related to the fact that the ascending colon has a very different physiological function than the descending colon? Or could it be related to the possibility that the internal milieu of these two regions of the colon are home to two different populations of microbiome organisms that secrete different factors that might affect the initiated adult stem cells of the two regions of the colon differently?

With the explosion of new physiological and pathological roles of the intestinal microbiome, one might be able to hypothesize that there exists in the right and left regions of the colon, products of normal colon physiology, as well as products of regional microbiome populations, that might explain differential “promoting” effects on the “initiated” stem cell

that triggers their mitogenesis and prevents their differentiation, leading to the resistant, flat type of tumors on the right side. On the other hand, differential normal physiological by-products of the left region or of the microbiome population of the left side could cause the clonal expansion of the partially-differentiated stem cell with expressed connexin genes. With expanding literature of the differential distribution of microorganisms in these two regions (Dougal et al. 2012; Nava et al. 2012; Flemer et al. 2016), it might be reasonable to predict the microbial toxins of these two regions might have differential effects on these two types of initiated but non-communicating stem cells.

In summary, it is speculated that the differences of the right- and left-sided origin of colorectal cancers’ sensitivity to anti-cancer treatments might be related to differential factors that could affect initiated stem cells’ ability to divide either symmetrically to form flat-type or embryonic-like lesions, which express Oct4A and ABCG2 genes, but not the connexin genes. On the other hand, if those factors in the left side of the colon affect the initiated stem cells to divide asymmetrically, then partially-differentiated or polyp-type lesions would be formed. These lesions probably do not express Oct4A or ABCG2 drug transporter genes, but do express some connexin gene. In addition, it has been shown that genetic and epigenetic changes in right and left colon cancer are significant different including microsatellite instability, methylation status and mutations, such as ras and raf, suggesting differential etiology of carcinogenesis and possible different initiated stem cells (Missiaglia et al. 2014).

To find a rationale for treatment strategies for lesions starting from the two different regions of the colon, it is proposed that, starting from Loewenstein’s original observation that cancer cells: (a) do not “contact inhibit” or have growth control; (b) do not terminally differentiate; and (c) do not apoptose properly under normal conditions, and (d) do not have functional gap junctional intercellular communication [Loewenstein and Kanno 1966], one should try to get these cancer cells to restore these properties. Also, since it has been shown that there are two kinds of cancer cells that lack deficient gap junctional intercellular communication (Trosko 2003), first, those that never expressed their connexin genes and are very embryonic-like and second, those that do have expressed connexin genes but these proteins are rendered non-functional by some posttranscriptional/posttranslational modification, be treated differently.

This, then, leads to the phenotypic differences in responses of the colon cancers of right and left regions to anti-cancer treatments. The cancer cells are very different, epigenetically, in a manner that makes them very different, phenotypically, to be treated as “cancer cells”. The same anti-cancer agent given to an “initiated” stem cell, which has its connexin genes, epigenetically repressed at the transcriptional level, will respond to this agent very differently that a cancer cell that is partially

differentiated because it has its connexin gene expressed, but is unable to communicate with a dysfunctional gap junction protein. From a theoretical perspective, to convert a cancer cell, which has no expressed connexin and no functional GJIC, to one that might be contact inhibited, differentiate or apoptose as a normal cell, one needs to transcriptionally activate the connexin gene. The use of agents, such as suberoylanilide hydroxamic acid (SAHA), a histone deacetylase (HDAC) inhibitor, (Ogawa et al. 2005), might be a strategy for the colorectal right located, embryonic-like undifferentiated tumors. On the other hand, treatment of tumor cells with expressed connexin genes but no functional GJIC because of a post-translationally-modified connexin protein might be treated with various inhibitors of oncogenes or agents that ameliorate the action of GJIC-inhibiting chemical promoters that could be rendering the gap junction protein from being transported to membrane or from forming gap junctions.

To test this hypothesis to explain this important observation of the location of the tumor in the colon that determines its sensitivity or resistance to treatment, there seems to be a straight forward testable series of experiments. Since stem cells and “cancer stem cells” express OCT4A, express drug transporter genes, such as ABCG2, but do not express connexin genes, the right-sided colorectal cancer tissues should be positive for these and other stem cell markers. The differentiated left-sided colorectal tumors, on the other hand, should express some differentiated colon genes, express connexin genes and gap junction proteins, but little or no OCT4A gene and no expressed drug transporter genes. Of course, the additional real problem of all tumors is the fact that tumors are mixtures of “cancer stem cells” and “cancer non-stem cells” (particularly, the polyp-type), as well as stromal and invasive immune cells. This complex micro-environment of many interacting soluble growth regulators, and cytokines, as well as insoluble extracellular matrix factors, must also be considered in both the design and interpretation of future experiments.

In conclusion, one critical hallmark of all normal cells (organ-specific stem cells; progenitor cells) is their ability to respond to growth suppression signals (secreted factors; extracellular matrix; gap junctions). On the other hand, cancer cells are characterized by their loss of growth control, inability to terminally-differentiate and dysfunctional apoptosis, which is correlated with their loss of gap junction function, either by the cancer cells having no transcription of any connexin gene or by having their expressed connexin protein made dysfunctional by posttranslational modification. Gap junction function can be modulated by many induced intra-cellular signaling pathways, e.g. phorbol ester- induced PK-C; src- induced signaling; DDT- altered Ca⁺⁺ signaling; etc.). Stem cells can be stimulated to divide either symmetrically or asymmetrically by many factors. Initiated stem cells appear to be able only to divide symmetrically by promoting, epigenetic agents that

can trigger various intra-cellular signals. In vivo, depending on the tissue/organ, various endogenous hormones, growth factors or cytokines could act as these agents to stimulate initiated stem cells. In addition, exogenous agents, such as pollutants, drugs, dietary factors, or toxins from the microbiome, could act to promote the symmetrical cell division of initiated organ-specific stem cells. In the case of the differences in response to cancer therapeutic agents, the resistance or sensitivity of the right- and left derived colorectal cancers might be related to the micro-environmental milieu of the adult stem cells’ response to different promoting factors in the ascending and descending colon.

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