Introduction

There are 1.4 million Americans diagnosed with cancer every year, with approximately 566,000 adults dying annually from the disease [1]. However, the factors that cause cancer are still under much debate. One popular explanation of tumorigenesis is the cancer stem cell (CSC) hypothesis. A consensus definition of the working group of the American Association of Cancer Research defines a CSC as a cell within a tumor that can self-renew and cause development of heterogeneic cells that make up the tumor [2]. The CSC model has been further refined into sequential development stages prior to tumorigenesis: a cell that becomes a tumor initiating cell (TIC) can give rise to a precancerous stem cell (pCSC), which may evolve into a CSC that can subsequently lead to the development of a population of malignant daughter cells [3].

According to the CSC hypothesis, the population of stem-like cells from which malignancies originate is extremely small. Researchers first demonstrated the frequency of stem-like cells in acute myeloid leukemia (AML) to be one cell out of 250,000. Moreover, investigators showed that a single undifferentiated cell was sufficient to initiate human AML in immuno-compromised mice, demonstrating that one stem-like cell could potentially result in the development of an entire tumor [4].

Since their identification in AML, stem cell-like populations have been found in solid tumors including glioblastomas [5], breast cancer [6], prostate cancer [7, 8], hepatocellular carcinoma [9, 10], colon cancer [11-13], pancreatic cancer [14], and cancers of the head and neck [15]. Based on the similarities between TICs and stem cells, researchers are currently working to determine the origin of CSCs. Investigators are seeking to determine whether CSCs arise from stem cells of embryonic origin [16], differentiated cells present in adult tissue that can acquire the ability to become undifferentiated and behave like stem cells [17, 18], or progenitor cells [19, 20], which are multipotent cells that
continually renew organ tissues. The CSCs that can lead to cancer may arise due to specific cellular aberrations, which can result from accumulated genetic modifications that include changes to the underlying genetic sequence and epigenetic abnormalities. In contrast to mutations in the genetic sequence of DNA, epigenetic changes occur beyond the level of DNA and alter the protein-DNA complex that forms chromosomes. One such epigenetic factor is parental imprinting [21].

Genes that are imprinted are expressed only from one parental allele. A loss of imprinting (LOI) results in two possible scenarios: the affected individual either has both alleles dually expressed due to activation of the silent allele or no expression due to suppression of the normally active allele [22]. Insulin-like growth factor 2 (IGF2), a gene whose end action is to stimulate general growth, is usually imprinted such that only the paternal allele is expressed. When LOI occurs, the maternal allele may also be expressed and some studies have, indeed, correlated LOI of IGF2 with increases in expression [21]. Further research on the imprinting of IGF2 could lead to a screening test with high sensitivity and specificity that would predict an individual’s future cancer risk. One promising, non-invasive example is that of screening via peripheral blood lymphocytes [21]. Many lines of research suggest that tumor initiation is connected to the imprinting patterns of IGF2, however the precise mechanisms remain to be elucidated [21].

**IGF2 imprinting regulation**

The regulation of gene imprinting occurs at CpG-enriched imprinting control regions (ICR), a differentially methylated region (DMR) that is variably methylated depending upon parent of origin during gonadal development and is involved in gene silencing. The ICR is normally utilized by the cell as a switch to permanently inactivate transcription, thereby allowing mono-allelic expression of a given gene to control gene dosage. Preservation of the ICR methylation status in all subsequent daughter cells is mediated by the maintenance methylase DNA methyltransferase (Dnmt1).

The importance of imprinting regulation can be demonstrated via human androgenotes, embryos with only a paternal genomic contribution, which develop early fatal embryonic malignancies, and parthenotes, embryos with only a maternal genomic contribution, which are characterized by severely retarded growth and subsequent gestational termination [23].

The ICR for the IGF2/H19 locus is located in the 5’ flanking region of the H19 gene and 90kb downstream of IGF2 (Figure 1). The ICR on the maternal allele is unmethylated, while the ICR on the paternal allele is methylated [24-28]. This methylation of the paternal allele ICR blocks the transcription factor CTCF-binding factor (CTCF) from binding and creating a physical barrier that stops downstream enhancers from augmenting IGF2 promoters. This effectively silences the maternal allele [29-31].

Mouse models have shown that CTCF binds to regions near the IGF2 promoter, as well as the ICR, and subsequently forms CTCF-CTCF dimers, creating an intrachromosomal loop [28, 32, 33]. The CTCF dimer then interacts with the SUZ12 (suppressor of zeste 12 homolog) domain of polycomb repressive complex 2 (PRC2) which methylates histone H3 lysine residue 27 (H3K27) causing silencing of the maternal allele [32]. Recently, Zhang et. al. elegantly demonstrated the role of CTCF in regulation by synthesizing decoy CTCF proteins. When introduced to cells, the decoys bind to the unmethylated ICR and IGF2 promoter but do not interact with SUZ12, thereby rendering Enhancer of zeste homolog 2 (EZH2), another part of PRC2, unable to methylate histone H3K27, resulting in reactivation of the imprinted allele [34]. Iderraabdullah et. al. looked at sequence content around the CTCF binding site and showed that that neither intact CTCF sites nor hypermethylation at the ICR is sufficient for maintaining paternal allele silencing, and sequences outside of the CTCF binding sites at the ICR are needed for silencing [35].

Thus, LOI of IGF2 may result from a variety of causes including: aberrant ICR methylation, a decreased expression of PRC2, a mutation of the ICR, or altered PRC2 H3K27 methylation. In contrast to the maternal allele, the paternal ICR is methylated, thereby blocking CTCF and PRC2 binding [29, 30]. One prominent example of epigenetic regulation of IGF2 gone awry is Beckwith-Wiedmann syndrome in which a maternally inherited microdeletion of two CTCF binding sites prevents PRC2 mediated methyla-
Role of IGF2 in cancer

Nearly every gene that is imprinted has been implicated as a regulator of embryonic, chorionic, or adult growth or metabolism [37]; IGF2 is a factor that is involved at all three stages. Blocking the action of IGF2 at birth has been shown to reduce birth weight to 60% below normal [38], while a two-fold increase in IGF2 expression results in a 131% increase in offspring growth [22, 39]. Circulating IGF2 ligand has been shown to regulate crosstalk between the WNT and IGF1R pathways, which can lead to activation of either the phosphoinositide 3-kinase (PI3K)-AKT or the Ras-MAPK (mitogen-activated protein kinase) pathways that control metabolism, growth, differentiation, and apoptosis [40].

While many of these pathways are upregulated in cancer, Vu et al. argue that elevated IGF2 levels alone may not be oncogenic [36]. Holm and colleagues claim their mouse tumor models of global loss of imprinting show IGF2 levels alone are insufficient for tumorigenesis. Even after restoration of normal imprinting, transformation cannot be abrogated in these mice [41]. Other murine models produced by Rogler and colleagues with IGF2 expression levels thirty fold higher than wild type were not sufficient to develop tumors until senescence. However, they were able to show constitutive overexpression of IGF2 did eventually lead to significantly higher rates of carcinomas in many tissues including hepato-cellular, squamous cell, and thyroid carcinomas, sarcomas, and a variety of other cancers [42]. The role of IGF2 in tumor initiation still appears to be uncertain and furthermore the mechanism of LOI of IGF2 mediated tumorigenesis may be completely separate from simply increasing IGF2 expression levels. Vu et al. hypothesize that LOI of IGF2 may instead globally impact relatively common chromatin looping structures resulting in aberrant long-range interactions [36]. At least one clinical scenario seems to support this conclusion: Russell-Silver syndrome is a disorder in which the ICR is unmethylated, thereby allowing
CTCF mediated repression of both alleles, though children with this condition do not have low circulating levels of IGF2 [43].

Significant associations have been made between patients with a family history of CRC and LOI of IGF2 [44]. One study showed a 500% increase in risk of adenoma formation in colonic mucosa among women who express LOI of IGF2 [45]. Sakatani et al. generated a murine model of LOI of IGF2 coupled with adenomatous polyposis and found a significantly increased rate of CRC in the mice [46].

In a review of the involvement of LOI of IGF2 in cancer, Cui et al. noted associations with a number of tumor types, including prostate cancer, breast cancer, lung cancer, colon cancer, and Beckwith-Wiedemann syndrome (BWS) [44]. Many tumor types have been associated

Table 1. Summary of recent studies of loss of imprinting of IGF2

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Frequency of LOI of IGF2</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>24/44 (55%)</td>
<td>[107]</td>
</tr>
<tr>
<td>Bladder</td>
<td>2/9 (22%)</td>
<td>[108]</td>
</tr>
<tr>
<td>Breast</td>
<td>0/9 (0%)</td>
<td>[109]</td>
</tr>
<tr>
<td>Cervical carcinoma</td>
<td>3/11 (33%)</td>
<td>[63]</td>
</tr>
<tr>
<td>Clear cell sarcoma of kidney (CCSK)</td>
<td>10/21 (48%)</td>
<td>[110]</td>
</tr>
<tr>
<td>Congenital mesoblastic nephroma (CMN)</td>
<td>6/14 (43%)</td>
<td>[111]</td>
</tr>
<tr>
<td>CRC</td>
<td>0/7 (0%)</td>
<td>[112]</td>
</tr>
<tr>
<td>Esophageal adenocarcinoma</td>
<td>70/149 (47%)</td>
<td>[66]</td>
</tr>
<tr>
<td>Gastric</td>
<td>30/52 (58%)</td>
<td>[67]</td>
</tr>
<tr>
<td>Gastric antrum</td>
<td>13/24 (54%)</td>
<td>[113]</td>
</tr>
<tr>
<td>Gastric corpus</td>
<td>24/38 (63%)</td>
<td>[69]</td>
</tr>
<tr>
<td>Hepatoblastoma</td>
<td>11/20 (55%)</td>
<td>[114]</td>
</tr>
<tr>
<td>CRC</td>
<td>26/95 (27%)</td>
<td>[68]</td>
</tr>
<tr>
<td>CRC</td>
<td>9/14 (64%)</td>
<td>[109]</td>
</tr>
<tr>
<td>Esophageal adenocarcinoma</td>
<td>14/44 (32%)</td>
<td>[57]</td>
</tr>
<tr>
<td>Gastric</td>
<td>18/40 (45%)</td>
<td>[61]</td>
</tr>
<tr>
<td>Gastric antrum</td>
<td>16/33 (48%)</td>
<td>[60]</td>
</tr>
<tr>
<td>Gastric corpus</td>
<td>10/30 (33%)</td>
<td>[61]</td>
</tr>
<tr>
<td>Hepatoblastoma</td>
<td>8/10 (80%)</td>
<td>[61]</td>
</tr>
<tr>
<td>Laryngeal squamous cell carcinoma LSCC</td>
<td>9/54 (17%)</td>
<td>[115]</td>
</tr>
<tr>
<td>Pancreatic endocrine</td>
<td>5/15 (33%)</td>
<td>[62]</td>
</tr>
<tr>
<td>(insulinoma)</td>
<td>6/15 (40%)</td>
<td>[116]</td>
</tr>
<tr>
<td>PBL of patients with personal history of CRC patients</td>
<td>5/5 (100%)</td>
<td>[117]</td>
</tr>
<tr>
<td>Serous epithelial ovarian</td>
<td>18/65 (28%)</td>
<td>[21]</td>
</tr>
<tr>
<td>Solitary fibrous tumor (SFT)</td>
<td>5/23 (22%)</td>
<td>[59]</td>
</tr>
<tr>
<td>Testicular germ cell</td>
<td>3/5 (60%)</td>
<td>[56]</td>
</tr>
<tr>
<td>Urothelial carcinoma of bladder (UCB)</td>
<td>16/39 (41%)</td>
<td>[118]</td>
</tr>
<tr>
<td>Wilms Tumor</td>
<td>7/17 (41%)</td>
<td>[119]</td>
</tr>
<tr>
<td>Wilms Tumor</td>
<td>2/6 (33%)</td>
<td>[36]</td>
</tr>
<tr>
<td>Wilms Tumor</td>
<td>17/40 (43%)</td>
<td>[120]</td>
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<tr>
<td>Wilms Tumor</td>
<td>14/35 (40%)</td>
<td>[121]</td>
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<tr>
<td>Wilms Tumor</td>
<td>31/59 (53%)</td>
<td>[53]</td>
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<tr>
<td>Wilms Tumor</td>
<td>20/28 (71%)</td>
<td>[122]</td>
</tr>
<tr>
<td>Wilms Tumor</td>
<td>23/33 (70%)</td>
<td>[123]</td>
</tr>
<tr>
<td>Wilms Tumor</td>
<td>2/36 (6%)</td>
<td>[124]</td>
</tr>
<tr>
<td>Wilms Tumor</td>
<td>11/32 (34%)</td>
<td>[55]</td>
</tr>
<tr>
<td>Wilms Tumor</td>
<td>16/97 (16%)</td>
<td>[54]</td>
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</table>

Abbreviations: CRC = colorectal carcinoma; PBL = peripheral blood lymphocytes
with imprinting aberrations, leading A.P. Feinburg to posit that LOI is considered to be one of the most abundant alterations in cancers [47]. We report here an update to the review by Cui et al. (Table 1).

BWS is a congenital, childhood syndrome characterized by overgrowth of some features and an increased proclivity to develop Wilms tumor, hepatoblastoma, and rhabdomyosarcoma. Sparago et al. were able to elucidate the mechanism of BWS by showing that microdeletions in the H19 DMR render two CTCF target sites incapable of binding CTCF and leading to hypermethylation of the DMR and bi-allelic expression of IGF2 [48].

In a comparison of the normal male prostate to prostate cancer, Fu et al. showed that LOI of IGF2 with increasing age is more extensive in men with cancer [49]. Another study by Bhusari and colleagues found that IGF2 levels increase with normal aging prostate seeming to implicate the LOI of IGF2 noted by Fu et al. as the culprit of increased expression. Additionally, LOI of IGF2 is not limited to the tumor itself but can be found two to fivefold higher in nearby and distant tissues (2-10mm), thus supporting the epigenetic progenitor model of cancer and corroborating work on intestinal tumors finding similar progenitor populations [50, 51].

LOI of IGF2 in Wilms tumor (WT), first identified by Rainer and colleagues [52], is considered the most common epigenetic or genetic aberration in these tissues. More recent work looking at other potential epigenetic alterations found that LOI of IGF2 in WT is the only statistically significant alteration, supporting the notion that epigenetic alterations in WT is not a global phenomenon but is in fact limited to IGF2 [53]. In a look at another common marker for WT's, Fukushima et al., demonstrated that LOI of IGF2 in WT does not occur with WT1 mutations and represents a distinct tumor class with separate histology, focal site, and other molecular aberrations [54]. At least one study attempted to define a relationship between imprinting status of IGF2 and expression levels and discovered that LOI of IGF2 correlated significantly with overexpression. Furthermore, the investigators identified two developmentally expressed genes, mesoderm-specific transcript homolog (MEST) and neuronatin (NNAT), which were also correlated with the imprinting status of the DMR of IGF2 [55].

In rare solitary fibrous tumors (SFT), Hajdu and colleagues showed that those with the highest expression all (5/5) showed LOI. Upregulation of IGF2 in SFT's can lead to Doee-Potter syndrome, a paraneoplastic disorder characterized by hypoglycemia due to excessive IGF2 release, which targets the IR-A receptor in SFTs [56].

In contrast to the positive correlation of LOI of IGF2 to protein expression levels in WT's, Zhao et al. found that esophageal adenocarcinoma (EADC) with LOI had significantly lower levels of IGF2 expression than normally imprinted tumors, implicating other mechanisms for controlling IGF2 levels. However, normal epithelia with LOI had increased expression levels of IGF2 relative to normally imprinted epithelia. Additionally, LOI of IGF2 in patients less than 65 years of age was associated with improved outcome following resection (disease free survival and overall survival). The high levels of IGF2 in normal tissues with LOI appear to lend further support to the epigenetic progenitor model [57].

Dammann et al. found that 91% of ovarian carcinomas had hypomethylation of the IGF2 DMR and 77% have hypermethylation of the CTCF binding site, with 73% having both aberrations. They were also able to correlate the methylation status of DMR with shortened relapse free survival [58]. In another study Murphy et al. noted LOI of IGF2 in serous epithelial ovarian cancers did not correlate with elevated IGF2 expression levels or hypomethylation of the IGF2 DMR [59].

In a study of a cohort of Asian patients with gastric cancer, Zuo et al. noted LOI of IGF2 in 48.5% and found that it was associated with higher grade tumors even after adjusting for confounding factors (P<.05). They were also able to find significantly higher levels of IGF2 in the blood of LOI patients (P<.01), however, did not find any difference in disease-free survival or overall survival [60]. Lu et al. were able to stratify tumor types based on their location with the stomach finding that gastric corpus tumors were more likely to have LOI of IGF2 than tumors of the gastric antrum in Chinese patients (80% vs 33%, n=40). Another seemingly confirmatory finding of the tumor aggressiveness noted in the study by Zuo et al. was the finding by Lu et al. that LOI of IGF2 in gastric cancers in Chinese patients was associated with lymph
LOI of IGF2 and the epigenetic progenitor model

node metastasis (OR 4.5, p = .038) [61].

Grbesa et al looked at laryngeal squamous cell carcinomas (LSCC) for IGF2 imprinting status as well as presence of Helicobacter pylori and found that 33% had bi-allelic IGF2 expression, finding that H. pylori presence had no effect on IGF2 imprinting patterns [62].

At least one investigator attempted to narrow down specific CpG islands and the effect of their imprinting status on IGF2 expression. Shetty et al. found a significant loss of methylation in exon 9 CpG cluster of IGF2 in breast tumor tissues when compared to controls. Similar to findings in WT’s expression levels as determined by IHC showed a mean twofold increase [63].

Imprinting status of IGF2 has been extensively studied in colorectal cancer with the hope of generating a noninvasive screening test [21]. Other avenues were explored by Ito et al. who discovered that hypomethylation of DMR is more prevalent than LOI, finding that 80% of colorectal cancers and 33% of breast cancers possesses the alteration and argue that the DMR methylation status is not a sufficient surrogate for LOI status. Additionally in their prospective study they found hypomethylation of the IGF2 DMR in peripheral blood in 10% of their cohort, with similar levels in controls and those who later went on to develop cancer, supporting the notion that DMR status is not predictive of development of cancer as previous authors suggested.

However, a highly significant difference in methylation status was found between colonic tumors and normal colonic tissue, indicating that screening of DMR methylation status in the tumors rather than peripheral blood may be an effective screening tool. They also found that DMR methylation decreased with age, indicating that environmental or age-related factors may bolster existing genetic predilection [64]. Miroglio et al. were able to find a significant difference in the methylation status of familial adenoma polyposus patients’ peripheral lymphocyte DMR2a when compared HNPCC or spontaneous CRC patients, showing that PBL may be useful for identifying malignancy in subtypes of CRC [65].

In addition to its potential use as a screening tool, LOI of IGF2 may be prognostic. In a prospective cohort study, Liou et al. showed that LOI of IGF2 was significantly associated with a poor prognosis in patients with stage IV colorectal cancer. However, higher plasma levels of IGF2 were associated with better overall survival, while higher tumor levels of IGF2 were correlated with a worse overall survival, indicating that overexpression of IGF2 in tumor tissues may not necessarily track the same levels in plasma [66]. LOI of IGF2 also seemed to correlate with relevant clinical and molecular characteristics. Ohta et al. found that a CRC that demonstrates tumors presenting with LOI of IGF2 are more likely to be found in the proximal colon. They also found that these patients have a significantly lower rate of PI3KCA mutations with similar rates of V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) and V-raf murine sarcoma viral oncogene homolog B1 (BRAF) mutations [67]. Sasaki et al. confirmed the finding of LOI tumors being more likely in the proximal colon and showed that the tumors were more consistent with poor differentiation and independence of microsatellite instability (MSI) [68]. Cheng et al. also found similar results, noting that MSI, KRAS, and BRAF mutation status were not correlated to IGF2 LOI or DMR hypomethylation. They also postulate that high levels of IGF2 transcription were the result of a mechanism unrelated to LOI [69].

The precise mechanism of LOI mediated tumorigenesis remains elusive. IGF2 misregulation is commonly found in many human cancers, making it difficult to parse out the effect of LOI of IGF2 from genomic misregulation as a whole. Future studies must attempt to clarify this relationship to further validate use of LOI as a biomarker and clinical target.

Conflicting models of tumorigenesis: the CSC model

Two primary models of tumorigenesis are currently under intense debate: the CSC model and the clonal evolution model. Advocates for the CSC model argue that there is a small subset of cells within the tumor that continuously divide, producing clones and terminally differentiated daughter cells [70, 71]. These daughter cells comprise most of the mass of the tumor but lack the potential for self-renewal, and thus they do not contribute to tumor propagation. Any genetic differences within the tumor result from anomalous mutations from the progenitor [72].
In contrast, proponents of the clonal evolution model postulate that the majority of tumor cells, not only a small population of cells as stated in the CSC model, are capable of self-renewal. Since there is no single progenitor cell, heterogeneity comes from clonal genetic and epigenetic differences among all tumor cells [73].

**Arguments for the CSC Model**

Supporters of the CSC model [14] point to the study in which Bonnet et al. transplanted human tumor cells into non-obese diabetic-severe combined immuno-compromised (NOD –SCID) mice. In the experiment, only an extremely small fraction of the cells, around the order of 1 in $10^4$ to $10^7$, retained the ability to cause leukemia. Proponents claim that this finding is evidence that the vast majority of the tumor mass is not capable of self-renewal and thus believe that only a few stem cells are able to propagate the tumor. The CSC argument is strengthened by Bonnet’s finding that tumors and metastases are highly heterogeneous [72]. These studies have been corroborated in other tumor investigations of solid tumors including gliobastomas [5], breast cancer [6], prostate cancer [7], hepatocellular carcinoma [9, 10], colon cancer [11-13], pancreatic cancer [14], and cancers of the head and neck [15].

The fact that tumors are made up of a variety of cell types points towards a multipotent progenitor stem cell population with multidifferentiative capacity. This is in contrast to a population of similar sister cells, which one would expect with the clonal evolution model. Other evidence comes from the persistence of metastatic tumors despite chemotherapies [74] or radiotherapies [75]: if the tumor was composed entirely of susceptible clonally related cells, then these therapies would result in obligate indefinite remission. However, these therapies result in subsequent relapse of the cancer, demonstrating that the therapy is successful in obliterating most of the CSC progeny but not the CSCs themselves. This CSC population then can re-populate the tumor via metastasis.

**Arguments against the CSC model**

Conflicting results, however, cast doubt on Bonnet’s original study. Pearce et al. found that 50% of AML tumor samples did not result in any tumor development, even when $10^7$ or $10^8$ cells were transplanted into NOD-SCID mice [76]. Furthermore, Quintana et al. demonstrated that up to 27% of human melanoma cells, when transplanted as individual cells, were sufficient to cause tumor formation in NOD-SCID mice, a percentage that is many orders of magnitude higher than Bonnet’s findings of 1 in $10^4$ to $10^7$ tumor cells [77]. Lastly, when experimentalists injected tumors derived from mice into experimental mice rather than using human-derived tumors, greater than 10% of the cells resulted in growth in the new host [73]. These findings have convinced a number of researchers to reject the CSC model in some types of cancers.

Opponents of the CSC hypothesis argue that xenotransplantation into the NOD-SCID mice may vastly underestimate the number of cells capable of self-renewal in human cancers. Critics of the murine NOD-SCID model, as shown in Bonnet et al., argue that the fault of the model may reside in a lack of homology between the human and murine signaling that is requisite for tumorigenesis, with only a vanishingly small number of tumor cells coincidentally adapting to the foreign environment of the mouse [73]. Further complications arise when one considers the vastly different environments between solid tumors and the blood: tumors in solid tissue must invade through tissue barriers, acquire vasculature, and metastasize. Some proponents of the CSC model concede the limitations of the NOD-SCID model but argue that Bonnet’s data shows that cancer cells that do grow in the xenotransplants are those that possess stem cell surface markers CD34+CD38, while the cells lacking these markers do not seed the mice [73].

**Resolution on tumorigenesis model conflict: support for the CSC model**

In normal organ homeostasis, a pool of stem cells continues to divide, providing one differentiated daughter cell and a remaining stem cell. The epigenetic progenitor model of cancer, a version of the CSC model, postulates that it is this stem cell population that becomes susceptible to tumorigenesis via epigenetic modifications [51]. This paradigm was first cohesively outlined in a Nature article by A. P Feinburg in which the evidence was outlined for such a model [47]:

- In vitro studies show reversibility of cancer
phenotype in solid and leukemia tumors

- Global epigenetic changes precede initial mutations in cancer
- Cloned mouse melanoma nuclei can differentiate into normal mice, indicating that epigenetic alterations, rescued by normal development, are responsible for the cancer phenotype
- Persistent disease after treatment with imatinib points towards the existence of a progenitor population not susceptible to the BCR-ABL blockade
- Mouse model studies show that IGF2 LOI in colonic epithelium produces an expanded progenitor cell population susceptible to genetic alterations leading to CRC

This model asserts that tumor formations do not occur through monoclonally directed initiating genetic mutations but via epigenetic polyclonal expansion before the formation of even a benign pre-cancerous lesion. Many lines of evidence support this model.

The same PCR2 complex that regulates IGF2 also regulates other embryonically expressed genes that are enriched for CpG islands. This indicates that tumor cells hijack the normal cellular machinery (involved in repressing transient developmental genes) for silencing tumor suppressor genes [78, 79]. Furthermore, early misregulation of imprinting machinery may lead to a very early predisposition to malignancy by making key tumor suppressor genes vulnerable to methylation and permanent silencing [79]. Early epigenetic inactivation of tumor progenitor cells may “addict” cancer cells to aberrant signaling pathways. This dependence may then allow tumor cells to acquire mutations that provide survival benefits to facilitate invasion and expansion [80].

Sakatani et al. further posited that through LOI of IGF2, these organ stem cells may undergo significant growth and hyperplasia, resulting in a larger population of undifferentiated cells that are more susceptible to tumorigenesis [46]. Additionally, LOI may also directly contribute to malignancy through the upregulation of the IGF2 receptor, which activates kinases that contribute to cell proliferation, gene expression, and cell survival [51].

Gao et al. further refine the CSC model into its own hierarchical development prior to tumorigenesis, starting with a TIC and leading to a pCSC, which can then evolve into a CSC that can give rise to a population of malignant daughter cells [3]. Based on findings presented by Gao et al., we support the conclusion that LOI of IGF2 represents an example of the epigenetic progenitor model of cancer via formation of TICs. Studies show that a large population of normal cells in peripheral blood and tissue in both Wilms' tumors and CRC also possess LOI, further supporting the notion that LOI occurs prior to malignancy [44]. Thus, it is our belief that imprinting defects lead to a globally or mosaically established predisposition to cancer by increasing survival and growth signaling in pre-cancerous progenitor cell populations, effectively creating CSCs.

Much of the focus in therapies directed at epigenetic misregulation has been focused on managing aberrant hypermethylation. Drugs like azacitidine and decitabine (nucleoside inhibitors of DNA methylation) show that DNA methylation is reversible and can produce favorable clinical outcomes [81]. These drugs have demonstrated efficacy in hematological disorders, especially myelodysplastic syndrome [82-86]. They have also been demonstrated to have broad efficacy including DNA damage, immune modulation, anti-apoptosis, formation of adducts between DNMTs and azanucleoside-substituted DNA working to re-express hypermethylated tumor suppressor genes [87-89]. Other potential therapies include histone deacetylase (HDAC) inhibitors, which can work to reverse HDAC aberrant silencing of genes. Two HDAC inhibitors, vorinostat and romidepsin, have been approved by the FDA for use in T-cell lymphoma [90-92]. One promising treatment modality appears to be combination of an HDAC inhibitor with a demethylating agent [93, 94].

**Application of the CSC Model: IGF2 LOI as a Potential Biomarker**

A study by Cui et al. of individuals with a familial or personal history of CRC showed that these individuals were significantly more likely to have LOI in colonic mucosal cells [21]. Interestingly, this study also noted the presence of LOI in peripheral blood lymphocytes (PBL), with each patient who displayed LOI in PBL also containing LOI in the colon. These findings are strongly suggestive of LOI as a constitutional epigenetic modification associated with initiation of CRC,
LOI of IGF2 and the epigenetic progenitor model

warranting consideration of LOI of IGF2 as a potential biomarker in CRC. Moreover, the discovery of LOI in PBL suggests that blood screening of lymphocytes could potentially detect CRC risk. In a recent review van Engeland et al. summarized the sensitivity of PBL testing as encouraging, ranging from 51% to 90% and specificity ranging from 70% to 100%. However, the authors advised caution in moving forward as, to date, no large prospective cohort studies comparing these markers to traditional colonoscopy or fecal occult blood testing (FOBT) have been published [95].

Recently, a prospective cohort study utilizing individual samples from the Nurses’ Health Study and Health Professional Follow-Up Study confirmed the use of LOI of IGF2 as a prognostic marker for CRC [96]. It has been known that LOI corresponds with hypomethylation in the differentially methylated region-O (DMR0). Baba and colleagues suggested that hypomethylation of the DMR0 may be a surrogate biomarker for LOI of IGF2 in colorectal cancer. Using bisulfite-PCR-pyrosequencing to detect methylation levels in the DMR0, they determined that colorectal neoplastic tissue has significantly less methylation at DMR0 when compared to normal, matched colorectal mucosa [96]. These results strongly suggest that methylation status, and therefore LOI of IGF2, of colorectal mucosa may be used as a diagnostic and prognostic risk marker for CRC. As evidence, CRC patients in the lowest quartile for methylation status were found to have significantly shorter survival periods and a higher overall mortality. This knowledge is particularly important because colorectal tumors displaying significantly hypomethylated epigenotypes could be identified early and targeted aggressively for treatment.

While the epigenotype of colorectal mucosal tissue appears to be a robust biomarker for CRC risk and prognosis, there is still considerable controversy about the use of PBL with LOI of IGF2 as an indicator for this purpose. A recent prospective study conducted by Kaaks et al. through the Northern Sweden Health and Disease cohort investigated the methylation status of two CpG sites in PBL with respect to CRC risk and prognosis [97]. In direct contrast to the findings of Cui et al., the results of this study showed no relationship between methylation status of DMR0 in PBL and CRC risk, a finding that may limit the potential use of LOI of IGF2 as a possible biomarker in blood. Additionally, researchers found that there was no association between LOI in PBL and onset of CRC within two years of blood sampling. In the interim, this conclusion may limit the diagnostic potential of LOI of IGF2, since blood screening of lymphocytes would be a considerably less invasive method in determining CRC risk when compared to screening of colorectal tissue.

It is worth noting that there are several distinguishing factors between these two disparate studies. First, Cui et al. performed a cross-sectional analysis, whereas Kaaks et al. investigated LOI in a prospective cohort manner. It is possible that LOI occurred around the same time as initiation of the neoplasm, which could account for the different results between the studies. However, this may have larger implications by casting doubt upon the validity of the epigenetic progenitor model of neoplastic survival. Another complicating factor is the population evaluated in each study. Cui et al. surveyed from a multiracial American population while those examined in the North Sweden cohort were entirely of Northern European descent. Therefore, given the populations studied, we maintain that the epigenotype of colorectal mucosal cells should be the preferred biomarker in CRC, while the role of LOI in PBL in CRC deserves further investigation to definitively resolve the importance of LOI in PBL as a potential biomarker.

Clinical application: targeted therapies for IGF2

LOI of IGF2 causes a bi-allelic expression of the gene, resulting in the overexpression of the IGF2 protein, an important molecule in intracellular signaling. IGF2 protein binds to two receptors, insulin-like growth factor type I receptor (IGF-1R) and insulin-like growth factor type II receptor (IGF-2R), which carry out distinct functions. IGF-1R activation is associated with upregulation of proliferative and anti-apoptotic agents such as AKT [98], while IGF-2R sequesters IGF2 protein for internalization and degradation [99]. Therefore, it appears that inhibition of IGF-1R activity and IGF-2R upregulation may be two distinct potential therapies for diverse tumors displaying overexpression of IGF2 protein.

Another study demonstrated that a diet lacking synthetic methyl donors, including folic acid,
vitamin B12, choline, and methionine, could cause LOI of IGF2 in murine models [100]. LOI of IGF2 and CSC’s increases DNA methylation, reducing the risk of hypomethylation and LOI [101]. Not all individuals who possess LOI of IGF2 develop cancer, as 10% of healthy individuals express this epigenetic malfunction at the IGF2 locus [102].

Interestingly, every solid tumor has been found to contain IGF-1R at the cell surface while simultaneously displaying a decrease of plasmalemmal IGF-2R [103]. In the same study, Mitsiades et al. investigated the effects of inhibition of IGF-1R on tumor cells by systemically administering an IGF-1R tyrosine kinase inhibitor, NVP-ADW742. They found that inhibition of IGF-1R by NVP-ADW742 not only suppresses tumor growth but also increases the efficacy of chemo-therapeutic agents administered concurrently. Encouragingly, inhibition of IGF-1R negatively affected tumor cell survival and spared normal cell survival, suggesting that while IGF-1R function is critical to neoplastic survival, it is not necessary for normal tissue endurance. The success of this experiment suggests that targeting the IGF-1R for inhibition may be a possible treatment in the future, especially for tumors already resistant to current antitumor medications.

Another prospective method for rescuing solid tumors from both LOI of IGF2 and the overexpression of IGF-2 protein is upregulation or administration of exogenous IGF-2 receptors. Using a mouse model expressing colorectal tumors with LOI, Harper et al. demonstrated that soluble high affinity IGF-2R effectively rescued the LOI phenotype associated with CRC [104]. This approach presents another novel treatment with possible clinical applications targeting tumors manifesting the LOI epigenotype.

While treatment for LOI of IGF2 with IGF-1R inhibitors or exogenous IGF-2Rs shows clinical promise, complete reversal of the LOI epigenotype offers a permanent treatment option. Using nuclear transfer techniques originally developed for stem cell cloning, correction of LOI has been achieved in the research laboratory [105]. Nuclei from tumor cell lines with LOI were transferred to enucleated cell lines with proven maintenance of normal imprinting, and the resulting reconstructed cells were evaluated for imprinting status. In every cell line, imprinting was recovered in the hybrid for several days. Upon further evaluation, Chen et al. determined that cytoplasmic trans factors were responsible for recovery and maintenance of imprinting in the reconstructed tumor cells [105]. Although nuclear transfer for clinical application is not currently a viable therapeutic strategy, it is our opinion that complete correction of the LOI epigenotype would be among the most desirable of the aforementioned therapies and that the potential for this therapeutic approach should be closely monitored in the future.

One similar alternative to nuclear transfer is the engineering of zinc-finger transcription proteins (ZFPs), which have been shown to reintroduce normal imprinting of IGF2 genes in a domain-specific manner [106]. Jouvenot et al. state that ZFPs can potentially be constructed more specifically to correct IGF2 dysfunction [106]. Interestingly, whether to utilize a therapeutic approach, such as either nuclear transfer or ZFPs, may be influenced by greater knowledge of cancer initiation and propagation. For instance, confirmation of the epigenetic progenitor model of cancer initiation would mean that only a small population of cells would need to be targeted for potential therapy.

**Conclusion**

While IGF2 can stimulate normal cellular growth, changes to IGF2 regulation and expression can produce deleterious physiological consequences. LOI of the IGF2 gene has been clearly demonstrated to have a strong association with the development of several forms of cancer, especially CRC. Recent research seems to demonstrate that this epigenetic modification, in fact, facilitates the process of tumor initiation, a finding that supports the epigenetic progenitor version of the CSC model of cancer formation.

LOI of IGF2 appears to be a useful biomarker and diagnostic tool for such forms of cancer as CRC. Subsequently, various therapies targeting this epigenetic modification have been shown to have initial success in treating cancer, such as inhibition of IGF-1R and the administration of exogenous IGF-2R in treating CRC. Especially promising is the inhibition of IGF-1R due to its lack of deleterious effects on normal tissue. Furthermore, engineered zinc-finger transcription proteins were found to successfully reintro-
duce normal imprinting of IGF2 alleles, offering a potential way to treat CRC and other cancers. Finally, the potential for nuclear transfer as a therapeutic approach must continue to be monitored.

Additional research may certainly help to confirm these assertions, especially regarding the extent to which LOI of IGF2 affects cancer development. Further studies in this area may include a larger, more diverse cohort of patients than those used by Cui et al. and Kaaks et al., which may help to elucidate their conflicting findings. Moreover, future research may also focus on the IGF2 protein, its two receptors, changes to their cellular signaling pathways based on epigenetic modifications to the IGF2 gene, and the extent to which these interactions affect different tissues. These findings would help to confirm the role of IGF2 in the development of other forms of cancer and potentially identify additional avenues for therapeutics.

Given the number of people affected by cancer, further research of IGF2 is warranted. Recent study findings support our assertion that LOI of IGF2 facilitates tumor initiation as per the epigenetic progenitor model of cancer formation and initiation. The development of treatment options that target this genetic change could lead to improved patient outcomes among patients diagnosed with cancers associated with LOI of IGF2.

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