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The role of pluripotency factors to drive stemness in gastrointestinal cancer

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ABSTRACT

A better molecular understanding of gastrointestinal cancers arising either from the stomach, the pancreas, the intestine, or the liver has led to the identification of a variety of potential new molecular therapeutic targets. However, in most cases surgery remains the only curative option. The *intra*tumoral cellular heterogeneity of cancer stem cells, bulk tumor cells, and stromal cells further limits straightforward targeting approaches. Accumulating evidence reveals an intimate link between embryonic development, stem cells, and cancer formation. In line, a growing number of oncofetal proteins are found to play common roles within these processes. Cancer stem cells share features with true stem cells by having the capacity to self-renew in a de-differentiated state, to generate heterogeneous types of differentiated progeny, and to give rise to the bulk tumor. Further, various studies identified genes in cancer stem cells, which were previously shown to regulate the pluripotency circuitry, particularly the so-called "Yamanaka-Factors" (OCT4, KLF4, SOX2, and c-MYC). However, the true stemness potential of cancer stem cells and the role and expression pattern of such pluripotency genes in various tumor cell types remain to be explored. Here, we summarize recent findings and discuss the potential mechanisms involved, and link them to clinical significance with a particular focus on gastrointestinal cancers.

Contents

1. Stem cell factors and cancer development

1.1. The "Yamanaka-Factors"

Stem cells are not only characterized by unlimited self-renewal, in fact, they also have the capacity to differentiate into virtually all tissue

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types. In 2006, Shinya Yamanaka was the first to successfully reprogram cells using four distinct factors, thereby generating induced pluripotent stem cells (iPSCs) from terminally differentiated fibroblasts. IPSCs can be established by the overexpression of four key transcription factors: OCT4, SOX2, KLF4, and c-MYC (OSKM) (Takahashi et al., 2007; Takahashi and Yamanaka, 2006; Maherali et al., 2007). Reprogramming of any somatic cell type can be achieved by initiating several synergistic processes. In the process of reprogramming, induced pluripotency elicits several transcriptional waves driven by c-MYC/KLF4 and OCT4/SOX2/KLF4. The expression levels of distinct pluripotency genes (alkaline phosphatase (AP), stage specific embryonic antigen (SSEA), Nanog and OCT4) increase step-wise (Brambrink et al., 2008), and upon achieving stable pluripotency levels, their DNA methylation patterns are changed (Polo et al., 2012). Nonetheless, the exact mechanisms of reprogramming still remain unclear. Obviously, the reprogramming factors reactivate an endogenous pluripotency circuitry by re-inducing the cells' capacity for unlimited growth without inducting genetic alterations, as it is frequently observed in cancer (Polo et al., 2012; Rais et al., 2013). It has been demonstrated that abbreviated (a slightly modified?) reprogramming factor expression pattern results in dysplasia and tumor formation *in vivo* (Ohnishi et al., 2014), thus suggesting that OSKM has an impact on epigenetic changes that are substantially involved in the regulation of cell growth and tumorigenesis. This observation is corroborated by the fact that iPSCs form teratomas upon implantation *in vivo* (Magnuson et al., 1982). Of note, human iPSCs develop teratoma more efficiently and faster than human embryonic stem cells (ESCs) (Gutierrez-Aranda et al., 2010; Avior et al., 2015).

1.2. Overexpression of OSKM leads to dysplasia and tumorigenesis in vivo

Several studies have assigned the OSKM factors to tumorigenesis. Abad et al. were the first to successfully reprogram *in vivo* by transiently inducing OSKM, resulting in teratoma formation, and detection of fully reprogrammed cells in various tissue types. Notably, the presence of the niche *in vivo* even allowed superior reprogramming to the totipotent state. However, this work is lacking any description regarding non-teratoma tumor formation (Abad et al., 2013). Intriguingly, further studies showed that partial or incomplete reprogramming induced particular tumor types *in vivo* (Ohnishi et al., 2014). To elucidate this time-dependent influence of OSKM overexpression, the authors used a doxycycline (dox) inducible system in embryonic stem cells with a polycystronic cassette encoding either four or three reprogramming factors. Upon doxycycline exposure, the chimeric mice exhibited time-dependent dysplasia and tumor formation in various tissue types.

In the kidney, OSKM-induced tumors bear features of a Wilms tumor, a common pediatric cancer. Interestingly, these tumors only display epigenetic alterations, as indicated by global changes in their DNA methylation patterns. Tumors originating from only partially reprogrammed iPSCs are readily reprogrammed into pluripotent cells by OSKM expression, thus suggesting a closer relationship to pluripotency than to the original somatic cell. Another study demonstrated, a short (<7 d) OSKM overexpression to lead to teratoma formation in the kidney and dysplasia in all tissue types, whereas prolonged overexpression resulted in irreversible tumor formation (Fig. 1). Interestingly, reprogramming of OSKM-induced tumors resulted in non-tumorigenic iPSCs that contributed to regular organ formation upon subsequent differentiation *in vivo*. This indicates that reprogramming with the Yamanaka factors primarily leads to epigenetic alterations, generating a “cancer-poised” but not yet “cancer-committed” state (Ohnishi et al., 2014). Furthermore, the authors addressed distinct roles of different pluripotency factors during tumorigenesis: while reprogramming with four (OSKM) or three factors (OKS) led to persistent dysplasia, the exclusion of OCT4 (KMS) initiated reversible dysplasia after removal of doxycycline . This observation is well in line with previous data, showing that ectopic expression of OCT4 blocks progenitor differentiation and subsequent dysplasia (characterized by an expansion of progenitor cells and increased transcriptional activity of β-catenin) in epithelial tissues (Ohnishi et al., 2014; Hochedlinger et al., 2005).

1.3. Unique properties of cancer stem cells

Similar to normal tissues, cancers comprise heterogeneous cell populations with distinct phenotypes, functions, and gene expression profiles (Marte, 2013). The phenotypic characteristics of some cancer cells, particularly of poorly differentiated to undifferentiated tumors, have been found to be quite similar to undifferentiated embryonic cells (Curry et al., 2015; Arsic et al., 2015; Cusulin et al., 2015; Tang et al., 2015).

Cancer stem cells (CSCs), one of the subgroups of tumor cells, share some of the critical properties with embryonic stem cells such as unlimited self-renewal, multi-lineage differentiation potential, and maintenance of the stemness state. Therefore, the impact of pluripotency factors, like OCT4, SOX2, KLF4 and c-MYC, in tumorigenesis seems obvious.

However, the key property of CSCs is their (virtually) exclusive tumorigenicity in secondary recipients *in vivo*. Along these lines, a subpopulation of migratory CSCs has been shown to be exclusively responsible for metastatic activity of pancreatic cancers (Hermann et al., 2007). Moreover, CSCs have been demonstrated to drive chemoresistance and subsequent tumor relapse (Cusulin et al., 2015; Tang et al., 2015; Saigusa et al., 2009; Todaro et al., 2007; Dean et al., 2005; Morrison et al., 2011).

In gastrointestinal cancers we and others have demonstrated that CSCs show elevated expression levels of genes associated with stemness and pluripotency, such as OCT4, Nanog, SOX2, and KLF4 (Lonardo et al., 2011; Sainz et al., 2015; Hermann et al., 2014), as well as increased activity of stemness-associated signaling pathways (Mueller et al., 2009; Hermann et al., 2013). We have been able to demonstrate that CSCs represent a challenging but very intriguing target for therapy, and that the combination of CSC-targeted therapies with standard chemotherapeutic treatment results in significantly greater response to therapy.

During different stages of malignant progression, several stemness-associated genes are specifically regulated in two mouse models of pancreatic cancer: in fully transformed cells expressing an oncogenic K-Ras mutation, treatment with nicotine results in upregulation of *Oct4* and also of other genes related to stemness in murine pancreas such as *Sox9*, *Hes1*, *ALDH1* and *Stat3*. Intriguingly, the de-differentiation of acinar tissue seems to be a critical step on the way to K-Ras-mediated transformation (Kopp et al., 2012). The key acinar regulators Gata6 and Mist1 play an essential role in maintaining acinar differentiation (Martinelli et al., 2013), but they are repressed by activation of the “OKSM-member” c-MYC, thus paving the way for malignant transformation by oncogenic Ras mutations (Hermann et al., 2014).

Patient tumors include a heterogeneous mix of subclones as a result of branching tumor evolution (Burrell et al., 2013). Multiple genetic and non-genetic factors drive tumor heterogeneity and contribute to distinct facets of malignancy: histone modification, DNA methylation, micro RNA and noncoding RNA expression, and genomic mutations as well as chromosomal aberrations (Meacham and Morrison, 2013). The mechanism of acquisition and maintenance of CSC properties are not entirely understood to date. However, their de-regulation of self-renewal may be a precondition for tumor development (Ricci-Vitiani et al., 2007; Vermeulen et al., 2008) (Fig. 2).

Further, both the presence as well as the amount of CSCs seem to be associated with a poor prognosis, respectively (Clevers, 2011). Many of the genetic alterations in cancer tilt the precise balance between cell differentiation and division, favoring the latter, ultimately resulting in a selective growth advantage, due to the fact that differentiating cells eventually become quiescent or die.