


## Perspective

# A ‘Goldmine’ for digging cancer-specific targets: the genes essential for embryo development but non-essential for adult life

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Cancer initiation and progression are usually triggered by protooncogene activation and/or tumor suppressor gene inactivation and promoted by further genomic and epigenetic alterations that reprogram cell gene expression, metabolism, proliferation, differentiation, and behavior. Overexpressed or mutation-activated tyrosine kinase receptors and their signaling components, such as HER2, EGFR, Src, RAS, PI3K, and AKT, steroid hormone receptors, such as estrogen receptor and androgen receptor, and other cell growth and cell cycle regulators induce carcinogenesis or promote cancer cell growth, survival, and progression. Accordingly, many therapeutic drugs have been developed and used to target these molecules for treating different cancers (Supplementary Table S1). Although these drugs have significantly improved cancer treatments, most oncogenic factors are also expressed in normal cells and required for normal physiological functions. Therefore, the drugs of anti-oncogenic factors also result in severe adverse effects on cancer patients. An ideal anti-cancer drug should specifically kill cancer cells without affecting normal cellular function,

which requires identifying targets essential for cancer cells but non-essential for normal cells. Importantly, these cancer-selective targets required for cancer cell survival may or may not be the classic oncogenes that have attracted extensive attention for drug development.

During embryonic development, cell proliferation, fate determination, and differentiation are controlled by temporal and spatial gene expression patterns. Many genes expressed at early embryonic growth, cell fate commitment, tissue-specific precursor cell proliferation, and differentiation stages play essential roles in supporting embryonic development. Knockout (KO) of any of these essential genes may result in embryonic lethality. However, once the embryonic development is completed, certain genes essential for embryonic development are no longer expressed or become non-essential for adult health.

It is known that embryogenesis and carcinogenesis share many similarities. For example, the highly proliferative feature and the cell cycle regulatory mechanisms of early embryonic cells and cancer cells are similar. The cell migration and invasion behaviors and the underlying regulatory mechanisms, such as reprogramming the cell plasticity through epithelial-to-mesenchymal transition (EMT), are similar between embryonic cells and cancer cells. The embryonic stem (ES) cells and cancer stem cells also share a number of similarities. The human embryonal carcinoma cells and ES

cells express a large set of genes at similar levels (Sparger et al., 2003). Cancers, particularly those poorly differentiated cancers including the high-grade ER-negative breast cancers, glioblastomas, and bladder carcinomas, also express ES cell gene signatures (Ben-Porath et al., 2008). Patients with cancers expressing a gene signature similar to that of stem cells exhibit poor overall survival (Riester et al., 2017). Similarities between embryonic cells and cancer cells are also found in DNA methylation patterns and pioneer transcription factors and architectural proteins that regulate chromatin organization (Larson and Yuan, 2012; Chiang et al., 2014; Dobersch et al., 2019). Because of these similarities, many genes essential for embryonic cell proliferation and survival are also expressed in cancer cells for supporting cancer cell proliferation and survival. Therefore, certain genes that are essential for both embryo and cancer cell growth and survival but non-essential for adult survival and general health may serve as cancer-specific/preferential targets for killing cancer cells with tolerable adverse effects on adult patients.

### Genes essential for embryogenesis may be non-essential for survival and general health in adulthood

KO mouse models have been extensively used to define the developmental and physiological functions of many genes. According to the data from the

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International Mouse Phenotyping Consortium (IMPC), among the 5327 genes studied in KO mouse models, individual KO of 1274, 509, and 3544 genes results in complete embryonic or neonatal lethality, partial lethality, and viable animal, respectively (IMPC, <https://www.mousephenotype.org/>; Dickinson et al., 2016). These results indicate that the functions of >1274 genes, which correlate with 1317 human orthologous genes, are absolutely required for embryo survival and development (Supplementary Table S2). However, the expression of many genes essential for embryo development is spatiotemporally regulated in accordance with embryo developmental stages and tissue differentiation states. After developmental events are accomplished, some of these essential developmental genes may become silenced or only expressed in a specific cell type or tissue. Although not many essential developmental genes have been tested in adult animals, several genes such as *Twist1* and *NR2F2* (also known as *COUP-TFI*) have been demonstrated to be essential for embryonic development but non-essential for adult life.

In the mouse embryo, *Twist1* is expressed in the mesoderm-derived tissues. Chen and Behringer (1995) have reported that all *Twist1*-null mouse embryos die in the uterus by embryonic day 11.5 (E11.5). Interestingly, we found that *Twist1* protein is only expressed in some of the mammary gland fibroblasts, the dermal papilla cells of the hair follicle, and some meninges membrane cells of the brain in adult mice (Xu et al., 2013). Inducible KO of *Twist1* in mice starting at 2 weeks of age or older ages does not cause lethality, and these inducible KO mice exhibit normal viability, activity, body weight, heart function, metabolism, reproductive function, and mammary gland development. The only phenotype we found is that these inducible KO mice show an extremely extended anagen phase of their hair follicles, which means that they do not lose their hair (Xu et al., 2013). These findings indicate that *Twist1* is a gene essential for embryo survival and development but non-essential for survival and general health of young

and adult mice after their developmental events are completed.

The *NR2F2* gene is broadly expressed in different cell types during the mouse embryonic development. Global KO of *NR2F2* in mice results in embryonic lethality before E11.5, which is mainly caused by defective angiogenesis and abnormal heart morphogenesis (Pereira et al., 1999). Conditional KO of *NR2F2* in a cell type-specific manner also demonstrated that *NR2F2* is required for prenatal or neonatal development of many other organs such as the eye, the stomach, and testicular Leydig cells (Takamoto et al., 2005; Qin et al., 2008; Tang et al., 2010). However, when *NR2F2* is globally knocked out in an inducible manner in adult mice, these mice survive well and do not show obvious defects as compared to age-matched wild-type mice. These inducible KO mice also have normal reproductive function (Qin et al., 2008). These findings indicate that *NR2F2* is another gene essential for embryo survival and development but non-essential for maintaining the normal life of adult mice.

In addition to *Twist1* and *NR2F2*, other genes known to be essential for embryo survival and development but non-essential for adult survival also include *Cripto-1*, *Noda1*, *Ror1*, *Birc5*, *Tbx2*, and *Trim28* (Rowley et al., 2004; Fukuda and Pelus, 2006; Strizzi et al., 2008; Abrahams et al., 2010; Rangel et al., 2012; Shabani et al., 2015; Kalyan et al., 2017; Rousseaux et al., 2018; Table 1). Up to date, only a small number of genes essential for embryo survival and development are examined in adult mice using inducible KO approach. We speculate that many more genes can be identified from the large pool of genes essential for development but non-essential for adulthood after the developmental process is completed.

#### Many genes essential for embryo growth and survival are also required for cancer cell growth and survival

A molecular target for cancer therapy should be required for cancer cell proliferation and/or survival, and inhibition or

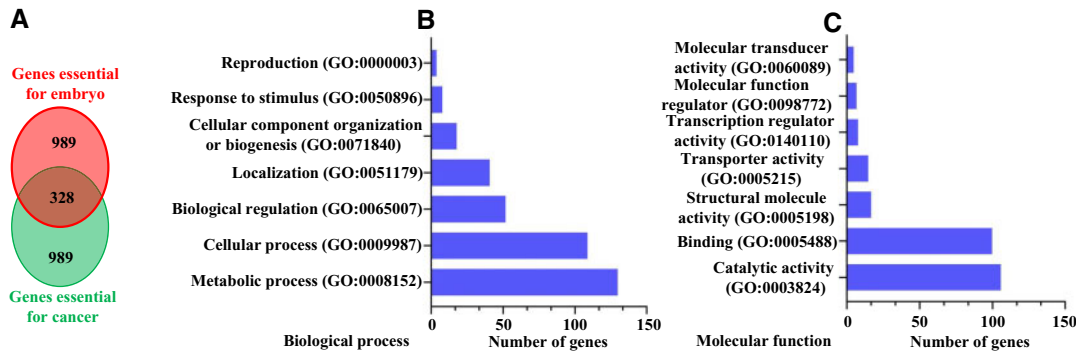
disruption of this target should suppress or kill cancer cells without killing normal cells. Using a genome-wide approach based on CRISPR/Cas9-mediated genetic mutations, Tsherniak and Hahn's group in Harvard University have examined individual genes required for proliferation and survival of 342 different cancerous cell lines and identified 1317 genes essential for these cancerous cells (Supplementary Table S2; Meyers et al., 2017). Coincidentally, the number of the human orthologous genes of the 1274 mouse genes essential for mouse embryo growth and survival is also 1317. When comparing these 1317 human orthologous genes identified from mouse KO studies with the 1317 genes essential for cancer cell proliferation and survival, we identified 328 genes required for both cancer cell and embryo growth and survival (Figure 1A; Supplementary Table S2). These 328 genes are mostly enriched in the metabolic process, cellular process, biological regulation, localization, and other biological processes (Figure 1B) and in the catalytic activity, binding, structural molecular activity, transporter activity, and other molecular functions (Figure 1C). Unfortunately, the physiological functions of these 328 genes have not been individually examined in adult mice by inducible KO strategy, and therefore, it is still unknown how many of these genes are non-essential for normal adult life. It is also important to point out that each of these 328 genes is required for the proliferation and survival of many different types of cancer cells. If focusing on one type of cancer cells, the number of essential genes for this cancer type would be much bigger than 328, which may explain why the several genes including *NR2F2* and *Twist1* in Supplementary Table S3 are not in the list of these 328 genes.

#### Genes essential for embryo and cancer cell growth and survival but non-essential for adult life can be the selective molecular targets for killing cancer cells in adult cancer patients

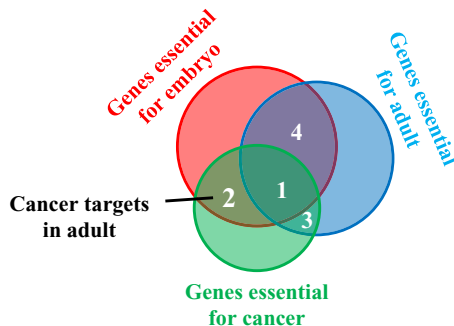
Among the 328 genes essential for both embryo and cancer cell growth and

**Table 1** Exemplary genes essential for mouse embryo development, non-essential for adult mouse viability, and expressed in cancer cells.

Genes	Mouse embryo		Adult mouse		Expression in cancers	References
	Expression	KO phenotype	Expression	KO phenotype		
<i>Twist1</i>	Mesoderm-derived tissues	Lethal by E11.5; defective cranial neural folds	Mammary gland fibroblasts, dermal papilla of hair follicle, and brain meninges	Healthy mouse with extended anagen phase of the hair follicle	Breast, bladder, pancreatic, prostatic, gastric, hepatocellular, and esophageal squamous cell cancers	Chen and Behringer (1995); Qin et al. (2012); Xu et al. (2013)
<i>NR2F2</i>	Mesenchyme tissues and developing vasculatures	Lethal by E11.5; defective angiogenesis and heart development	Uterus, liver, stomach, mammary gland, kidney, prostate, heart, lung, and brain at low levels	No obvious abnormal phenotype	Breast, prostatic, colon, and ovarian cancers	Xu et al. (2015)
<i>Trim28</i>	Oocyte and early embryo	Lethal by E5.5; defective preimplantation	Expressed in 324 organs, with highest level in trachea	No obvious abnormal phenotype	Breast, lung, liver, gastric, and prostatic cancers	Rousseaux et al. (2018)
<i>Tbx2</i>	Mesenchyme in lung, craniofacial, and posterior ectodermal ridge	Lethal between E10.5 and E14.5; defective heart development	Heart, lung, kidney, ovary, and melanocyte lineage cells	No reported abnormal phenotype; human <i>TBX2</i> not linked to any genetic disease	Breast, pancreatic, liver, and bladder cancers and melanoma	Rowley et al. (2004); Abrahams et al. (2010)
<i>Nodal</i>	Early inner cell mass	Lethal after gastrulation; defective primitive streak formation	No expression in almost all of the adult tissues	No reported abnormal phenotype	Breast, prostatic, pancreatic cancers and melanoma	Strizzi et al. (2008); Kalyan et al. (2017)
<i>Cripto-1</i>	Gastrulation stage, nascent primitive streak, and mesoderm	Lethal by E7.5; defective gastrulation and germ layer formation	Very low in normal adult tissues except mammary gland	No reported abnormal phenotype; human <i>CRIPTO-1</i> not linked to any genetic disease	Breast, colon, pancreas, lung, ovary, stomach, gall bladder, cervix, testicle, skin, and bladder cancers	Strizzi et al. (2008); Rangel et al. (2012)
<i>Ror1</i>	Head mesenchyme	Embryonic lethal due to respiratory distress and cyanosis	Low levels in nervous, circulatory, respiratory, digestive, urogenital, and skeletal systems, eyes, nose, and ears	No reported abnormal phenotype; no congenital disease linked to <i>ROR1</i> mutations in human	Leukemia, lymphoma, multiple myeloma, and solid tumors such as breast cancer	Shabani et al. (2015)
<i>Birc5</i>	Distal bronchiolar epithelium of the lung and neural crest-derived cells	Lethal by E4.5; defective microtubule formation	Mostly in thymus and placenta	No reported abnormal phenotype	Esophageal, lung, ovarian, breast, colorectal, bladder, gastric, prostatic, pancreatic, laryngeal, uterine, hepatocellular, and renal cancers, melanoma, and soft tissue sarcomas	Fukuda and Pelus (2006)



**Figure 1** Identification of common genes essential for both mouse embryo development and cancer cell proliferation. (A) The distribution of genes that are essential for both embryo development and cancer cell proliferation and only essential for either embryo development or cancer cell proliferation. (B and C) GO enrichment analyses of the biological processes (B) and molecular functions (C) for the 328 common genes essential for both embryo development and cancer cell proliferation. The top seven terms with enriched common genes are presented.



**Figure 2** A hypothetical model for the relationships among genes essential for human embryo development, adult health, and cancer cell proliferation. Group 1 genes are essential common genes for embryo development, adult life, and cancer cell proliferation. Group 2 genes are essential for embryo development and cancer cell proliferation but non-essential for adult life, which are the potential cancer-specific molecular targets. Group 3 genes are essential for both adult life and cancer cell proliferation, but non-essential for embryo development. Group 4 genes are essential for both embryo development and adult life but non-essential for cancer cell proliferation.

survival, only several proteins including BRD4, CDK9, HDAC3, and mTOR have specific inhibitors that are approved by FDA or enrolled in clinical trials as therapeutic reagents. Several BRD4 small-molecule inhibitors including JQ1, I-BET762, and BMS-986158 also bind to other BET family proteins and induce drug resistance of cancer cells (Duan et al., 2018). Another problem is that suppression of BRD4 by inducible RNAi expression in adult mice disrupts tissue homeostasis in multiple organs and induces intestinal stem cell loss, suggesting that BRD4 is not non-essential

for adult health and thus not an ideal selective target for cancers (Bolden et al., 2014). The CDK9 inhibitors including Dinaciclib, Alvocidib, and AT7519, the HDAC3 inhibitors including Panobinostat and Belinostat, and the mTOR inhibitors including Everolimus and Temozolimus can inhibit cancer cell proliferation and survival, but they may also have adverse effects on normal health in adult cancer patients, since these proteins are involved in a number of normal physiological processes in adult. However, it has not been studied whether these several genes are essential

for adult life by using inducible KO mouse models.

*Twist1* and *NR2F2* are two exemplary genes essential for embryo and important for certain types of cancers but non-essential for adult life. *Twist1*-mediated EMT in breast cancer cells plays an important role in promoting cancer cell migration, invasion, metastasis, dedifferentiation toward cancer stem-like cells, and resistance to therapies (Yang et al., 2004; Mani et al., 2008; Qin et al., 2012; Xu et al., 2017). *NR2F2* strongly augments PTEN loss-induced prostate cancer progression and metastasis by overriding TGF- $\beta$ -dependent cell proliferation checkpoint though inhibiting SMAD4-dependent gene expression (Qin et al., 2013). Therefore, it would be logical to predict that specific and potent inhibitors for *Twist1* and *NR2F2* could benefit breast cancer and prostate cancer treatments, respectively.

Further experiments can be carried out to identify potential cancer-specific targets from the 328 genes essential for embryo and cancer. Each of these genes can be knocked out in adult mice in an inducible manner to select those genes non-essential for adult life. The expression profiles of the selected genes can be examined and compared in mouse and human embryo and adult tissues to determine whether these genes have similar spatial and temporal expression

patterns in mouse and human. Since the initial screening for the genes essential for cancer cells was performed in 2D cell cultures that lack the 3D tumor environment, the selected genes can be knocked out in human cancer cell lines, which can be compared with control cells in the 3D organoid culture system to determine whether the selected genes are required for the formation and growth of the cancer cell-derived organoids. The selected genes also can be knocked down in the tumor cells of patient-derived xenograft (PDX) mouse models to determine their requirements in PDX tumor growth. Finally, small-molecule inhibitors can be developed for the yielded candidate gene products and tested in all the aforementioned cancer models. Importantly, the off-targeting effects of a developed inhibitor on organs and normal physiological functions in adult life can also be evaluated in adult mice with inducible KO of the gene target for the inhibitor.

### Conclusion remarks

In summary, many genes essential for mouse embryo growth and survival but non-essential for adult mouse life have been identified by gene KO studies. Although these genes identified in mice may be different from those in human, the general concept should be the same, i.e. the genes essential for human embryo and cancer cell survival and growth but non-essential for adult human survival and health should be the ideal molecular targets for cancer therapy in adult cancer patients (Figure 2). With the identification of these cancer cell-specific molecular targets regardless of their roles in cancer initiation and progression, effective drugs of these targets can be developed to selectively kill cancer cells with minimal adverse effects on adult patients.

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