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 repress genes essential for both embryonic cell proliferation, fate determination, and differentiation. 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 (Sperger 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 embryonic 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
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 | |
| Twist1 | 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) |
| NR2F2 | 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) |
| Trim28 | 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) |
| Tbx2 | 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 TBX2 not linked to any genetic disease | Breast, pancreatic, liver, and bladder cancers and melanoma | Rowley et al. (2004); Abrahams et al. (2010) |
| Nodal | 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) |
| Cripto-1 | 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 CRIPTO-1 not linked to any genetic disease | Breast, colon, pancreatic, lung, ovary, stomach, gall bladder, cervix, testicle, skin, and bladder cancers | Strizzi et al. (2008); Rangel et al. (2012) |
| Ror1 | 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 ROR1 mutations in human | Leukemia, lymphoma, multiple myeloma, and solid tumors such as breast cancer | Shabani et al. (2015) |
| Birc5 | 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) |
gesting that BRD induces intestinal stem cell loss, suggesting homeostasis in multiple organs and expression in adult mice disrupts tissue suppression of BRD. et al., 2018, other BET family proteins and induce 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 Temsirolimus 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, de-differentiation 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-β-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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References

Abrahams, A., Parker, M.I., and Prince, S. (2010). The T-box transcription factor Tbx2: its role in development and possible implication in cancer. IUBMB Life 62, 92–102.

Ben-Porath, I., Thomson, M.W., Carey, V.J., et al. (2008). An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors. Nat. Genet. 40, 499–507.

Bolden, J.E., Tasdemir, N., Dow, L.E., et al. (2014). Inducible in vivo silencing of Brd4 identifies potential toxicities of sustained BET protein inhibition. Cell Rep. 8, 1919–1929.

Chen, Z.F., and Behringer, R.R. (1995). twist is required in head mesenchyme for cranial neural tube morphogenesis. Genes Dev. 9, 686–699.

Chiang, J.H., Cheng, W.S., Hood, L., et al. (2014). An epigenetic biomarker panel for glioblastoma multiforme personalized medicine through DNA methylation analysis of human embryonic stem cell-like signature. OMICS 18, 310–322.

Dickinson, M.E., Renniken, A.M., Ji, X., et al. (2016). High-throughput discovery of novel developmental phenotypes. Nature 547, 508–514.

Dobersch, S., Rubio, K., and Barreto, G. (2019). Pioneer factors and architectural proteins mediating embryonic expression signatures in cancer. Trends Mol. Med. 25, 287–302.

Duan, Y., Guan, Y., Qin, W., et al. (2018). Targeting Brd4 for cancer therapy: inhibitors and degraders. Medchemcomm 9, 1779–1802.

Fukuda, S., and Pelus, L.M. (2006). Survivin, a cancer target with an emerging role in normal adult tissues. Mol. Cancer Ther. 5, 1087–1098.

Kalyan, A., Carneiro, B.A., Chandra, S., et al. (2017). Nodal signaling as a developmental therapeutics target in oncology. Mol. Cancer Ther. 16, 787–792.

Larson, J.L., and Yuan, G.C. (2012). Chromatin states accurately classify cell differentiation stages. PloS One 7, e31414.

Mani, S.A., Guo, W., Liao, M.J., et al. (2008). The epithelial–mesenchymal transition generates cells with properties of stem cells. Cell 133, 704–715.

Meyers, R.M., Bryan, J.G., McFarland, J.M., et al. (2017). Computational correction of copy number effect improves specificity of CRISPR–Cas9 essentiality screens in cancer cells. Nat. Genet. 49, 1779–1784.

Pereira, F.A., Qiu, Y., Zhou, G., et al. (1999). The orphan nuclear receptor COUP-TFII is required for angiogenesis and heart development. Genes Dev. 13, 1037–1049.

Qin, J., Tsai, M.J., and Tsai, S.Y. (2008). Essential roles of COUP-TFI in Leydig cell differentiation and male fertility. PloS One 3, e3285.

Qin, J., Wu, S.P., Creighton, C.J., et al. (2013). COUP-TFI inhibits TGF-β-induced growth barrier to promote prostate tumorigenesis. Nature 493, 236–240.

Qin, Q., Xu, Y., He, T., et al. (2012). Normal and disease-related biological functions of Twist1 and underlying molecular mechanisms. Cell Res. 22, 90–106.

Rangel, M.C., Karasawa, H., Castro, N.P., et al. (2012). Role of Cripto-1 during epithelial-to-mesenchymal transition in development and cancer. Am. J. Pathol. 180, 2188–2200.

Riester, M., Wu, H.J., Zehir, A., et al. (2017). Distance in cancer gene expression from stem cells predicts patient survival. PloS One 12, e0173589.

Rousseaux, M.W., Revelly, J.P., Vazquez-Velez, G.E., et al. (2018). Depleting Trim28 in adult mice is well tolerated and reduces levels of α-synuclein and tau. eLife 7, e36768.

Rowley, M., Grothey, E., and Couch, F.J. (2004). The role of Tbx2 and Tbx3 in mammary development and tumorigenesis. J. Mammary Gland Biol. Neoplasia 9, 109–118.

Shabani, M., Naseri, J., and Shokri, F. (2015). Receptor tyrosine kinase-like orphan receptor 1: a novel target for cancer immunotherapy. Expert Opin. Ther. Targets 19, 941–955.

Spegher, J.M., Chen, X., Draper, J.S., et al. (2003). Gene expression patterns in human embryonic stem cells and human pluripotent germ cell tumors. Proc. Natl Acad. Sci. USA 100, 13350–13355.

Strizzi, L., Postovit, L.M., Margaryan, N.V., et al. (2008). Emerging roles of nodal and Cripto-1 from embryogenesis to breast cancer progression. Breast Dis. 29, 91–103.

Takamoto, N., You, L.R., Moses, K., et al. (2005). COUP-TFI is essential for radial and anteroposterior patterning of the stomach. Development 132, 2179–2189.

Tang, K., Xie, X., Park, J.I., et al. (2010). COUP-TFs regulate eye development by controlling factors essential for optic vesicle morphogenesis. Development 137, 725–734.

Xu, M., Qin, J., Tsai, S.Y., et al. (2015). The role of the orphan nuclear receptor COUP-TFI in tumorigenesis. Acta Pharmacol. Sin. 36, 32–36.

Xu, Y., Lee, D.K., Feng, Z., et al. (2017). Breast tumor-specific knockout of Twist1 inhibits cancer cell plasticity, dissemination, and lung metastasis in mice. Proc. Natl Acad. Sci. USA 114, 11494–11499.

Xu, Y., Xu, Y., Liao, L., et al. (2013). Inducible knockout of Twist1 in young and adult mice prolongs hair growth cycle and has mild effects on general health, supporting Twist1 as a preferential cancer target. Am. J. Pathol. 183, 1281–1292.

Yang, J., Mani, S.A., Donaher, J.L., et al. (2006). Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell 117, 927–939.