Tumor and Stem Cell Biology

β-Catenin/POU5F1/SOX2 Transcription Factor Complex Mediates IGF-1 Receptor Signaling and Predicts Poor Prognosis in Lung Adenocarcinoma

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Abstract

Cancer stem-like cells (CSC) are crucial in tumor initiation and progression; however, the underlying mechanism for the self-renewal of cancer cells remains undefined. In the study, immunohistochemical analysis of specimens freshly excised from patients with lung adenocarcinoma showed that high expression of insulin-like growth factor I receptor (IGF-IR) in lung adenocarcinoma cells was positively correlated with the expressions of cancer stem cell markers CD133 and aldehyde dehydrogenase 1 family member A1 (ALDH1A1). IGF-IR activation enhanced POU class 5 homeobox 1 (POU5F1) expression on human lung adenocarcinoma stem-like cells (LACSLCs) through PI3K/AKT/GSK3β/β-catenin cascade. POU5F1 could form a novel complex with β-catenin and SOX2 to bind Nanog promoter for transcription to maintain self-renewal of LACSLCs, which was dependent on the functional IGF-IR. Genetic and pharmacologic inhibition of IGF-IR abrogated LACSLC capabilities for self-renewal and tumorigenicity in vitro. In an in vivo xenograft tumor model, knockdown of either IGF-IR or POU5F1 impeded tumorigenic potentials of LACSLCs. By analyzing pathologic specimens excised from 200 patients with lung adenocarcinoma, we found that colocalization of highly expressed IGF-IR with β-catenin and POU5F1 predicted poor prognosis. Taken together, we show that IGF-IR-mediated POU5F1 expression form a complex with β-catenin and SOX2 is crucial for the self-renewal and oncogenic potentials of LACSLCs, and the integrative clinical detection of the expressions of IGF-IR, β-catenin, and POU5F1 is indicative for predicting prognosis in the patients of lung adenocarcinoma.

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Introduction

Lung cancer is the leading cause of cancer-related human deaths with increasing incidence and less than 14% of the overall 5-year survival rate worldwide (1). Non–small cell lung cancers (NSCLC), which can be subdivided into adenocarcinoma, squamous cell, and large cell carcinomas, account for approximately 85% of all lung cancers (2). Lung adenocarcinoma is the most common histologic type of NSCLC, and most patients encounter treatment failures owning to the highly invasive and metastatic phenotype.

Accumulating evidence has been emerging that tumors contain a very small subpopulation of cancer stem-like cells (CSC) or tumor-initiating cells (TIC; refs. 3, 4). CSCs, similar to somatic stem cells, possess a variety of unique biologic properties including self-renewal, propagation and production of differentiated progeny, expressions of specific cell surface markers and stem cell genes, and usage of common signaling pathways and stem cell niche (5–7). CSCs differ from normal stem cells in their tumorigenic capacities for cancer initiation, recurrence, metastasis, and therapy resistance. Although the existence of CSCs in human lung cancer has been previously reported (3, 8, 9), the regulation of self-renewal and stemness maintenance for lung CSCs in the initiation of lung adenocarcinoma remains unclear.

IGF-IR is critical in malignant transformation and promoting cell survival, motility, angiogenesis, and metastasis of cancer cells (10). POU class 5 homeobox 1 (POU5F1, also known as octamer-binding transcription factor 4, Oct-4) is pivotal in the regulation of self-renewal and pluripotency of both somatic stem cells and CSCs (8, 11). However, the correlation of their expressions in the prognosis of patients...
with lung adenocarcinoma and the mechanism underlying the regulation of self-renewal and stemness maintenance for lung adenocarcinoma stem-like cells (LACSLC) remain obscure.

In the present study, we examined the correlation of IGF-IR expression with CSLC markers CD133 and aldehyde dehydrogenase 1 family member A1 (ALDH1A1) in specimens excised from patients with lung adenocarcinoma. An in vitro sphere culture system to isolate and enrich LACSLCs was established to explore the underlying molecular mechanisms. We then analyzed the expression of POU5F1 and IGF-IR as well as its downstream molecule β-catenin and the clinicopathologic correlations with a tissue microarray (TMA) containing 200 lung adenocarcinoma specimens. We show that IGF-IR–mediated POU5F1 expression to form a complex with SOX2 is crucial for the self-renewal of LACSLCs in the initiation of lung adenocarcinoma, and the expressions of IGF-IR, β-catenin, and POU5F1 predict poor prognosis in patients with lung adenocarcinoma.

Materials and Methods

Patients and tissue microarray
Pathologic specimens of 200 patients with primary lung adenocarcinoma, who received surgical resection in Thorax Department of Cancer Center, Sun Yat-Sen University (Guangzhou, China) between February 1994 and January 1998, were included. Tumor grades were defined according to the criteria of World Health Organization. The tumor–node–metastasis (TNM) status of all lung adenocarcinomas was assessed according to the criteria of the International Union Against Cancer (12). The clinicopathologic characteristics of the patients were summarized in Supplementary Table S1. The TMA was constructed as previously described (13) and the study was approved by the Medical Ethics Committee of Cancer Center of Sun Yat-Sen University.

Cell lines and cell culture
Human lung adenocarcinoma cell lines A549 and H358 were obtained from the American Type Culture Collection and were authenticated by the Cell Bank of Type Culture Collection of Chinese Academy of Science. Tumor sphere culture was conducted as described previously (14). Cells were cultured in the serum-free medium (SFM) composed of DMEM/F12 (Gibco), basic fibroblast growth factor (bFGF; 20 ng/mL; Upstate), EGF (20 ng/mL; Sigma-Aldrich), and B27 supplement (20 μL/mL; Life Technologies). Isolation and characterization of LACSLCs are described in Supplementary Figs. S1 and S2.

cDNA microarray and data analysis
Human genome microarray analysis was conducted by CapitalBio Corporation. Genes were determined to be differentially expressed when logarithmic gene expression ratios were more than 2-fold different and the P values were less than 0.05. For data validation, mRNA levels of the interested genes were analyzed by quantitative real-time PCR (qRT-PCR). All target genes and primer sequences used for validation will be provided upon request. To examine genes that might be systemically altered, both Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathways Analysis and Gene Set Enrichment Analysis (GSEA; ref. 15) were used for pathway analysis.

Self-renewal and colony formation assay
Tumor spheres generated by LACSLCs were dissociated into single-cell suspension. Cells were cultured in the stem cell media to obtain second- and third-generation spheres. Floating spheres and the total cell numbers were counted under light microscopy. For colony formation, adherent monolayer cells and LACSLCs were dissociated into single-cell suspension. The cells were plated into 4.8-mm dishes at a density of 200 or 400 cells per well in Dulbecco’s Modified Eagle Medium (DMEM) with 10% FBS. The plates were further incubated for 14 days at 37°C with 5% CO2 until colonies were visible. The colonies were stained with 0.01% crystal violet and counted under inverted microscopy.

Immunofluorescence and immunohistochemistry
Immunohistochemistry (IHC) of tissue array and tumor xenografts was conducted by using streptavidin–biotin peroxidase complex method. For immunofluorescence staining, monolayer cells and LACSLCs were attached to poly-L-lysine–coated coverslips in DMEM containing 10% FBS for 4 hours and subsequently fixed in 4% paraformaldehyde for 20 minutes (16). The detailed procedures are described in Supplementary Materials and Methods. In addition, freshly frozen human surgical biopsy specimens excised from 8 patients with lung adenocarcinoma were used in IHC staining.

Chromatin immunoprecipitation
Chromatin immunoprecipitation (ChIP) was conducted as previously described (17). The primer pairs used for PCR to amplify Nanog promoter region containing POU5F1-binding element were: 5’-ACCTAGAAGATTTTGTTGTGGT-3’ (sense) and 5’-AATTCTCAGTTACTCCGTCTAC-3’ (antisense; ref. 18).

Statistical analysis
All experiments were carried out at least 3 times with triplicate samples. Data were presented as the mean ± SEM. Statistical analysis was conducted using SPSS13.0 software. Statistically significant difference was assessed by one-way ANOVA followed by multiple mean comparisons by Student–Newman–Keul’s test. For univariate survival analysis, survival curves were obtained by using Kaplan–Meier method, and comparisons were made by using log-rank test. Multivariate survival analysis was conducted on all parameters found significant in univariate analysis by using Cox proportional hazards regression model. Statistical difference was considered significant if P values were less than 0.05.

Results

Aberrant expression of IGF-IR is positively associated with CSLC marker in lung adenocarcinoma specimens
To elucidate whether IGF-IR expression is associated with CSLCs in lung adenocarcinomas, the protein levels of IGF-IR and 2 CSLC markers for lung adenocarcinomas, CD133 and ALDH1A1, were examined in the tissue samples from patients with lung adenocarcinoma. As shown in Fig. 1, high level of IGF-IR in lung adenocarcinoma cells was positively correlated with increased levels of CD133 and ALDH1A1 in 8 fresh lung
regardless of their identity), de

with 0, 1, and 2 of the abovementioned 2 unfavorable factors (biomarkers, with lung adenocarcinoma were strati

diamidino-2-phenylindole (DAPI; blue). Higher magni

S3). We further observed an overall signi

fi

adenocarcinoma specimens (Fig. 1A and Supplementary Fig. S4). More

over, the combined expression of double-positive for IGF-IR and ALDH1A1 presents the prediction for the patient’s worse survival outcome (Fig. 1D).

IGF-IR signaling sustains the self-renewal and proliferation of LACSLCs

It remains elusive whether IGF-IR and its downstream signaling pathway impact LACSLCs. We established a stable sphere culture system for isolating and enriching LACSLCs from lung adenocarcinoma cell lines A549 and H358 for microarray analysis and mechanism studies. Ontology categories of microarray data (P < 0.001) were summarized on the basis of Gene Ontology database (Supplementary Tables S2–S4) and confirmed by qRT-PCR (Supplementary Tables S5 and S6). Genes that were significantly altered in LACSLCs were functionally categorized into signaling pathways in KEGG database. Top 20 signaling pathways were correlated with differentially expressed genes in LACSLCs (P < 0.02), and insulin/IGF-IR signaling pathway was most predominant according to the P values that were computed by GSEA and Ingenuity Pathways Analysis (Fig. 2A and Supplementary Fig. S5A), qRT-PCR and Western blot analysis as well as immunofluorescence staining confirmed that the mRNA and protein levels of the genes related to IGF-IR/insulin receptor substrate 1 (IRS-1) axis such as IGF-IR, IRS-1, and β-catenin were significantly upregulated in LACSLCs (Fig. 2B and C and Supplementary Fig. S3B–S5E).

We next assessed the effects of IGF-IR activation on the self-renewal of LACSLCs by examining their capabilities of tumor sphere-forming and colony formation. IGF-I increased the amount and size of the newly formed spheres as well as the cell numbers, which was inhibited by IGF-IR inhibitor picro-

podophyllin (PPP; Fig. 2D and Supplementary Fig. S6A), indicati

gating that the effect of IGF-I on LACSLCs was IGF-IR-dependent. Furthermore, IGF-I also induced colony formation by LACSLCs, which was significantly decreased by PPP (Fig. 2E and Supplementary Fig. S6B), LACSLCs infected with lentivirus containing short hairpin RNA (shRNA)–targeting IGF-IR yielded less numbers of smaller spheres and reduced colony formation (Fig. 2F and Supplementary Fig. S6C and S6D) with a significant decrease in the capacity to form offspring spheres (Fig. 2G), indicating a lower frequency of stem-like cells derived from IGF-IR-knockdown spheres.

The role of IGF-IR in the self-renewal of LACSLC in vivo was revealed by examining tumorigenic potentials of IGF-IR-knockdown LACSLCs. We found that tumors derived from LACSLCs with IGF-IR–shRNA yielded much smaller sizes than those with scrambled shRNA (Fig. 2H and Supplementary Fig. S6E). Tissues derived from the tumors formed by IGF-IR-knockdown LACSLCs displayed low density of microven

Adenocarcinoma specimens (Fig. IA and Supplementary Fig. S3). We further observed an overall significantly positive association of upregulated expression of IGF-IR with increased CSLCs marker ALDH1A1 in a TMA containing 200 cases of lung adenocarcinoma tissues (Fig. 1B and C). The average percent-

age of lung adenocarcinoma cells that were stained positive with ALDH1A1 in IGF-IR high-expression group (ALDH1A1 47.1%) was significantly higher than that in IGF-IR low-expres-

sion group (ALDH1A1 27.7%; P < 0.01). The association between the expression of IGF-IR and the clinicopathologic features of this lung adenocarcinoma cohort was illustrated in Supplementary Table S1. Kaplan–Meier analysis showed that high expressions of IGF-IR and ALDH1A1 in lung adenocarcinomas were correlated closely with poor disease-specific survival (DSS) of the patients (Supplementary Fig. S4). Moreover, the combined expression of double-positive for IGF-IR and ALDH1A1 presents the prediction for the patient’s worse survival outcome (Fig. 1D).
diminished invasion into muscle layers (Supplementary Fig. S6F). In addition, IGF-IR-knockdown decreased the numbers of proliferating cells in the tumors as indicated by Ki-67 staining (Supplementary Fig. S6F). Tumors derived from IGF-IR-knockdown LACSLCs expressed increased levels of lung-specific differentiation markers such as cytokeratin 8 (CK8) and cytokeratin (CK18) and contained fewer numbers of IGF-IR-, phosphorylated IRS-1-β-catenin- and POU5F1-positive cells (Supplementary Fig. S6F).

IGF-IR/IRS-1 axis activates POU5F1 via PI3K/AKT/GSK3β pathway

Stimulation of IGF-IR activates PI3K/AKT pathway to increase POU5F1 in somatic stem cells (19). We observed that the phosphorylations of IGF-IR, IRS-1, AKT, and glycogen synthase kinase 3β (GSK3β) in LACSLCs were increased (Fig. 3A). GSK3β, which regulates Wnt/β-catenin pathway (20), is one of well-known targets by PI3K/AKT pathway. When GSK3β was phosphorylated, the expression of both β-catenin and POU5F1 was increased (Fig. 3B). PPP inhibited the phosphorylation of IGF-IR, IRS-1, AKT, and GSK3β, thereby reduced the expression of β-catenin and POU5F1 (Fig. 3B–D). IGF-I further increased GSK3β phosphorylation and the expressions of β-catenin and POU5F1 (Fig. 3D). Although IGF-I activated both PI3K/AKT/GSK3β and Ras/Raf/MEK/ERK pathway, phosphoinositide 3-kinase (PI3K)-specific inhibitor LY294002 but not mitogen-activated protein/extracellular signal–regulated kinase (MEK)–specific inhibitor PD98059 decreased expressions of β-catenin and POU5F1 as well as GSK3β phosphorylation (Fig. 3D and E and Supplementary Fig. S7). Furthermore, IGF-IR-knockdown decreased IGF-IR expression in LACSLCs, leading to the reduction in the phosphorylations of
IGF-IR knockdown reduces the formation and function of β-catenin/POU5F1/SOX2 complex in LACSLCs

POU5F1 is needed for the stemness maintenance of CSLCs, but its relation to β-catenin and SOX2, 2 important factors for the maintenance of CSC phenotype, have not been addressed. We found that POU5F1 interacted with β-catenin and SOX2 to form a complex (Fig. 4A and B) and bound Nanog promoter to initiate transcription (Fig. 4C). Confocal scanning microscopy showed β-catenin/POU5F1/SOX2 complexes preferentially localized in the nuclei of LACSLCs (Fig. 4B). IGF-IR knockdown significantly reduced the interaction of the complex with Nanog promoter (Fig. 4D), resulting in the decreased expressions of Nanog and downstream genes such as Esrrb, Foxd3, and Sall1 (Fig. 4E). After knockdown of either Pou5f1, Sox2, or β-catenin by siRNA or shRNA, the mRNA levels of Nanog, Esrrb, Foxd3, and Sall1 in LACSLCs were significantly reduced (Fig. 4E). Thus, our data highlight the importance of IGF-IR signaling in regulating POU5F1 transcriptional function.

High expressions of IGF-IR, POU5F1, and β-catenin are correlated with the survival of lung adenocarcinoma patients

Immunohistochemical analysis revealed that IGF-IR expression concurred with the levels of POU5F1 and β-catenin (Supplementary Fig. S8). We analyzed the expressions of IGF-IR and POU5F1 as well as β-catenin and the clinicopathologic correlation with lung adenocarcinoma specimens from 200 patients (Fig. 5A). High expressions of IGF-IR, β-catenin, and POU5F1 were respectively found in 156 (78.0%), 120 (60.0%), and 121 (60.5%) cases. The associations between the expression of IGF-IR, β-catenin, and POU5F1 and the clinicopathologic features were illustrated in Supplementary Table S1. Further correlation analyses showed that the expressions of IGF-IR, β-catenin, and POU5F1 were positively correlated with each other (Fig. 5B). Thus, our data highlight the importance of IGF-IR signaling in regulating POU5F1 transcriptional function.

Discussion

CSCs, a subpopulation of cancer cells that possess unique survival capabilities and distinct stem cell properties (21), play a critical role in cancer initiation, progression, and recurrence (3, 22). IGF-IR plays key roles in malignant transformation and...
promotes the survival, invasion, and metastasis of tumor cells (23–26). Previous studies have reported that high expression level of IGF-IR was associated with poor survival of patients with various types of cancer (10). Up to date, limited data exist on clinical significance of IGF-IR expression in lung cancer, and the association between IGF-IR and poor prognosis remains controversy (27, 28). In this study, we found that IGF-IR could mediate the formation of a novel β-catenin/POU5F1/SOX2 complex in LACSLCs to play a crucial role in self-renewal of lung cancer cells and predict poor prognosis.

Our data firstly showed the activation of IGF-IR signaling in the lung adenocarcinoma spheres enriched for CD133- and ALDH1A1-positive CSLCs, that is, LACSLCs. These cells exhibit stem cell-like functional characteristics with high tumorigenicity (29–33). Microarray-assisted pathway analysis revealed that IGF-IR and IRS-1 were highly expressed, and insulin/IGF-IR signaling pathway was ranked as the top one used by LACSLCs for their advantage. Activated IGF-IR becomes a tyrosine kinase to phosphorylate adaptor molecule IRS-1, leading to the activation of downstream signaling pathways such as PI3K/AKT and Ras/Raf/MEK/ERK pathways (34, 35). IGF-IR signaling has been reportedly involved in the biologic properties of normal and neoplastic stem cells (19, 24, 36). We hereby found that IGF-IR activation promoted LACSLC self-renewal, whereas its inhibition/knockdown significantly reduced tumor sphere and colony formation and abrogated the tumorigenic activity of LACSLCs by inhibiting their proliferation and promoting differentiation.

Our findings of which deprivation of IGF-IR reduced β-catenin and POU5F1 expression show a role of IGF-IR-mediated signaling in the self-renewal of LACSLCs. Adult stem cell self-renewal is a tightly controlled process governed by the developmental pathways such as Wnt, Notch, and Hedgehog (4, 5, 11, 22, 37). Activation of Wnt/β-catenin pathway is important for the development of pluripotency (20, 38), and GSK3β, which is regulated by PI3K/AKT pathway, has also emerged as a key molecule to regulate self-renewal (39). Our data that IGF-IR activation regulates expressions of both β-catenin and POU5F1 by modulating IRS-1, AKT, and GSK3β indicate a cross-talk between IGF-IR signaling and Wnt/GSK3β/β-catenin pathway, and are consistent with previous reports that PI3K/AKT/GSK3β pathway can regulate both normal and malignant progenitor cells (20, 40). The IGF-IR/IRS-1 axis–mediated PI3K/AKT and Raf/MEK/ERK activation is linked with the phenotype and function of cancer cells (41, 42). PI3K/AKT and Raf/MEK/ERK pathways synergize to stimulate cell proliferation, and their dysregulation is associated with malignant transformation of cells (43, 44).

POU5F1 expression induced by IGF-I is sensitive to the inhibition of PI3K/AKT but not Raf/MEK/ERK pathway,
suggesting that PI3K/AKT pathway is critical in the self-renewal of LACSLCs. PI3K/AKT-mediated GSK3\(\beta\) phosphorylation controls nuclear translocation of \(\beta\)-catenin to functionally interact with POU5F1 (45, 46). A POU5F1-centered transcriptional network orchestrated by other transcription factors including SOX2 and Nanog regulates the self-renewal of stem cells (47–50). We show that POU5F1 interacts with \(\beta\)-catenin and SOX2 to form a novel complex and enhance Nanog expression, suggesting that IGF-IR/IRS-1/PI3K/AKT/GSK3\(\beta\) cascade-mediated regulation of POU5F1 and Nanog may act in a feedback loop to sustain the self-renewal of LACSLCs.

Clinically, we found the detection of IGF-IR–dependent expressions of both \(\beta\)-catenin and POU5F1 was of great importance for predicting prognosis in lung adenocarcinoma. We observed a positive linkage among expressions of IGF-IR, \(\beta\)-catenin, and POU5F1, and that higher expression of each of the 3 proteins was significantly correlated with reduced patient survivals. Moreover, we identified for the first time that simultaneous expression of 3 molecules predicts a tendency of shortest patient survivals. Thus, the IHC of IGF-IR, \(\beta\)-catenin, and POU5F1 expressions can be a practical protocol for analysis of lung adenocarcinoma outcomes.

In summary, the present study provides a new insight into the mechanisms underlying IGF-IR signaling in CSLCs. Our findings reveal that IGF-IR/IRS-1/PI3K/AKT/GSK3\(\beta\) cascade-mediated regulation of POU5F1 and formation of \(\beta\)-catenin/POU5F1/SOX2 complex are important for the retention of the self-renewal and tumorigenicity of LACSLCs. Moreover, the combined expression of triple-positive for IGF-IR, \(\beta\)-catenin, and POU5F1 shows the worst prediction for the patient’s poor survival rate \((P < 0.001)\).

Table 1. Correlation of the expressions of IGF-IR, \(\beta\)-catenin, and POU5F1 in 200 cases of lung adenocarcinoma

| Cases | IGF-IR | | | POUSF1 | | |
|-------|--------|--------|--------|--------|--------|
| \(\beta\)-Catenin | | | | | |
| Low | 80 | 24 (30.0%) | 56 (70.0%)\(^a\) | 80 | 41 (51.3%) | 39 (48.8%)\(^c\) |
| High | 120 | 20 (16.7%) | 100 (83.3%) | 120 | 38 (31.7%) | 82 (68.3%) |
| POU5F1 | | | | | |
| Low | 79 | 24 (30.4%) | 55 (69.6%)\(^b\) | 80 | 41 (51.3%) | 39 (48.8%)\(^c\) |
| High | 121 | 20 (16.5%) | 101 (83.5%) | 120 | 38 (31.7%) | 82 (68.3%) |

\(^aP = 0.026; \chi^2\) test.\n\(^bP = 0.021; \chi^2\) test.\n\(^cP = 0.006; \chi^2\) test.
**Table 2. Multivariate Cox regression analysis for the DSS of 200 patients with lung adenocarcinoma**

<table>
<thead>
<tr>
<th>Factors</th>
<th>HR (95% confidence interval)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-IRa</td>
<td>1.247 (0.793–1.961)</td>
<td>0.339</td>
</tr>
<tr>
<td>β-Cateninb</td>
<td>1.754 (1.217–2.529)</td>
<td>0.003</td>
</tr>
<tr>
<td>POUSFIa</td>
<td>1.338 (0.928–1.928)</td>
<td>0.118</td>
</tr>
<tr>
<td>Agec</td>
<td>1.290 (0.918–1.811)</td>
<td>0.142</td>
</tr>
<tr>
<td>Tumor gradec</td>
<td>0.491 (0.371–0.650)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T statusd</td>
<td>1.349 (0.960–1.896)</td>
<td>0.084</td>
</tr>
<tr>
<td>N statuse</td>
<td>1.837 (1.279–2.640)</td>
<td>0.001</td>
</tr>
<tr>
<td>M statuse</td>
<td>1.258 (0.688–2.299)</td>
<td>0.456</td>
</tr>
</tbody>
</table>

aLow expression vs. high expression.

bAge ≤ 59 vs. > 59 y.

cG1 vs. G2 vs. G3.

dT1-2 vs. T3-4.

*eN0 vs. N1-3.

fM0 vs. M1.

IGF-IR, β-catenin, and POUSFI are useful as integrative prognostic biomarkers for clinical management of patients with lung adenocarcinoma.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**References**

β-Catenin/POU5F1/SOX2 Transcription Factor Complex Mediates IGF-I Receptor Signaling and Predicts Poor Prognosis in Lung Adenocarcinoma

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