Pan-cancer Multi-Omics Identifies SKP2 as a Marker of Immune Exclusion with In Vivo Validation in Osteosarcoma

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Significance & Hypothesis

Immune checkpoint inhibitors have shown limited activity in osteosarcoma, underscoring the need for actionable biomarkers and sensitizers. SKP2, the substrate-recognition subunit of the SCF E3 ubiquitin ligase complex, is classically linked to proliferation. We hypothesized that SKP2 restrains anti-tumour immunity and contributes to an immune-cold tumour immune microenvironment (TIME).

Innovation

This study integrates pan-cancer multi-omics profiling with in vivo validation in an immunocompetent genetically engineered osteosarcoma mouse model to establish SKP2 as a marker of immune exclusion. It provides direct experimental evidence that the SKP2-associated immune-cold phenotype is reversible upon Skp2 loss, supporting SKP2 inhibition as a rational immunotherapy-sensitizing strategy.

Approach (Materials & Methods)

TCGA and GTEx transcriptomes (33 tumour types), protein expression (Human Protein Atlas), and genomic alterations (cBioPortal) were integrated. TIME associations were assessed by correlating SKP2 with ESTIMATE ImmuneScore, immune-cell infiltration signatures, and acellular immune components (MHC molecules, chemokines and chemokine receptors; TISIDB). Single-cell expression was analysed using TISCH and CancerSEA. Immunotherapy relevance was evaluated across eight immune checkpoint inhibitor cohorts using TIDE (six melanoma and two renal cancer cohorts). For in vivo validation, multiplex immunofluorescence (PANO 7-plex) was performed on autochthonous osteosarcoma tissues from Osx1-Cre;$Rb1^{lox/lox}$;$p53^{lox/lox}$ (DKO) and Osx1-Cre;$Rb1^{lox/lox}$;$p53^{lox/lox}$;$Skp2^{-/-}$ (TKO) mice (n=3 per group) using F4-80, CD3, CD8A, granzyme B, PD-1 and PD-L1 (Figure 1).

Results

SKP2 expression was significantly upregulated in 26 of 33 tumour types, with genetic alterations dominated by copy-number amplification (highest in NSCLC 9.59%, BLCA 8.27%, ESCA 7.56%). Across most tumour types, higher SKP2 correlated with lower ImmuneScore and reduced infiltration of cytotoxic immune subsets (CD8+ T, NK, NKT and γδ T cells), alongside decreased expression of MHC molecules and chemokines. In melanoma cohorts, high SKP2 was significantly associated with poor responses in three datasets and showed a consistent trend toward worse overall survival in three additional datasets; in contrast, higher SKP2 was associated with better outcomes in two renal cancer cohorts. In osteosarcoma, DKO tumours displayed an immune-cold TIME, whereas TKO tumours showed increased infiltration of macrophages (F4-80+), T cells (CD3+), CD8+ T cells (CD8A+), cytotoxic T cells (CD8A+ granzyme B+), NK cells (CD8A− granzyme B+), and exhausted CD8+ T cells (CD8A+ PD-1+) (Figure 2).

Discussion

SKP2 is a multi-omics marker of immune exclusion across solid tumours and is linked to reduced checkpoint benefit in melanoma, with tumour-type-specific exceptions. In vivo validation in an immunocompetent osteosarcoma model

indicates that this immune-cold phenotype is reversible upon Skp2 loss, supporting SKP2/SCF–SKP2–CKS1 inhibition as a rational stratification and combination strategy for immunotherapy in musculoskeletal sarcomas.

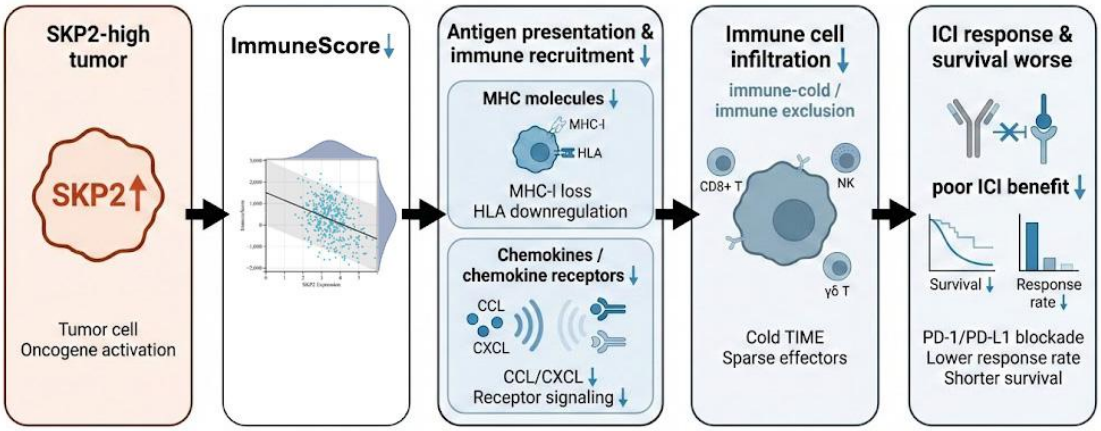

Figure 1 High SKP2 is linked to lower ImmuneScore, reduced MHC/chemokine expression, decreased immune-cell infiltration, and worse ICI outcomes.

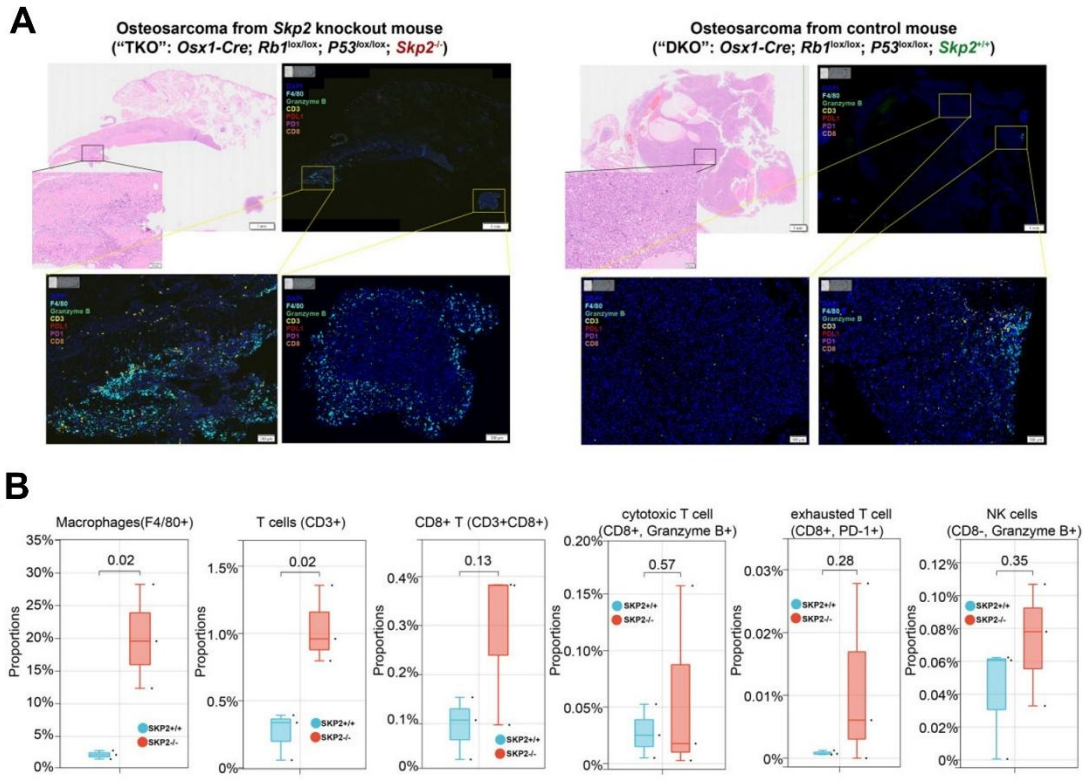

Figure 2. mIF of osteosarcoma tissues derived from Skp2 knockout and control transgenic mouse models. **A** Example of whole sections output from Skp2 knockout osteosarcoma (left) and control osteosarcoma (right) using mIF staining technology. DAPI, F4-80, granzyme B, CD3, PD-L1, PD-1, and CD8 staining, with the colors indicated, are shown on a whole section, with magnified insets from a representative donor. Scale bars, 1 mm and 100 µm. **B** Summary plot of various immune cell abundances from Skp2 knockout (n = 3) and control transgenic (n = 3) mouse

models. P -values < 0.05 are considered to be statistically significant.