tp53 deficiency causes a wide tumor spectrum and increases embryonal rhabdomyosarcoma metastasis in zebrafish

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Figure 1. Homozygous \( \text{tp53}^{\text{del/del}} \) zebrafish spontaneously develop a wide range of tumor types. (A) \( \text{tp53} \) genomic locus and CG1 \( \text{tp53}^{\text{del/del}} \) allele. TALEN arms were designed to target the 5' and 3' genomic sequence of \( \text{tp53} \) (red). (B–M) CG1 \( \text{tp53}^{\text{del/del}} \) zebrafish develop leukemia (B–D), Angiosarcoma (E), MPNST (F–J) and Germ Cell tumors (K–M). Figure 1 continued on next page.
Figure 1 continued

angiosarcoma (E–G), MPNSTs (H–J), and germ cell tumors (K–M). Whole animal images (B,E,H,K), hematoxylin/eosin (H and E) stained sections (C,D,F, G,I,L,M), and immunohistochemistry for Sox10 (J). Blast-like leukemia cells predominate in the kidney marrow and efface the renal tubules (black arrow, (D)). (N) Tumor incidence in CG1 tp53<sup>del/del</sup> zebrafish (n = 134). (O) Quantitation of tumor types that form in CG1 tp53<sup>del/del</sup> mutant zebrafish by 55 weeks of life based on histology review (n = 51). (P–S) kRAS<sup>G12D</sup>-induced embryonal rhabdomyosarcoma (ERMS) generated in CG1 tp53<sup>del/del</sup> zebrafish. Whole animal bright field and GFP-epifluorescence overlap images (P and Q, respectively). H and E stained sections revealed features consistent with human ERMS (R,S). Scale bars equal 12.5 mm in whole animal images and 100 μm in histology images.

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Figure 1—figure supplement 1. *tp53<sup>del/del</sup>* zebrafish survive at expected Mendelian ratios, lack Tp53 protein expression, and are resistant to irradiation-induced cell death. (A) Survival of animals by genotype from a heterozygous *tp53<sup>wt/del</sup>* in-cross. *tp53* homozygous wild-type (*wt/wt*), heterozygous (*wt/del*), and homozygous (*del/del*) mutant fish. (B) Western blot analysis at 24 hr post-fertilization (hpf) whole embryos. Actin is used as a loading control. (C–D) TUNEL staining performed on *tp53<sup>wt/wt</sup>* and *tp53<sup>del/del</sup>* embryos following gamma-irradiation at 24hpf (16 Gray) and fixation at 30hpf. Whole embryos images are shown for representative animals of each genotype (C). Quantification of TUNEL-positive cells within 1000 micron<sup>2</sup> area. Regions where cells were counted are outlined by the white boxes in panel C. p<0.001 by Student’s T-test.

DOI: https://doi.org/10.7554/eLife.37202.003
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Figure 1—figure supplement 2. Tumor latency in \textit{tp53}^{del/del} zebrafish. Designation as assessed by histology review.
DOI: https://doi.org/10.7554/eLife.37202.004
Figure 2. *tp53*del/deltumors efficiently transplant into syngeneic CG1 strain zebrafish. (A–E) A primary *tp53*del/del MPNSTs that formed in the eye transplanted orthotopically into the periocular space (A–C) or into the peritoneum of CG1-strain recipient fish (D–E). Intraperitoneal injection (i/p). (F–I) *tp53*del/del*Tg(ubi:GFP)*-positive angiosarcoma. Primary tumor-bearing fish (F–G) and transplanted animal (H–I). (J–R) *tp53*del/del*Tg(ubi:GFP)*-positive leukemia. Primary leukemia (J–K) and transplanted leukemia shown at 20 days post-transplantation (L–R). Whole kidney marrow was isolated from leukemia-engrafted fish and analyzed by FACS (N–O). (N) Forward and side scatter plot of whole kidney marrow of unlabeled CG1 host animal to assess *ubi:GFP*-positive *tp53*del/del leukemia cells following transplantation. (O) Analysis of GFP+ *ubi:GFP*-positive *tp53*del/del leukemia cells following FACS. Purity was >90%. (P–R) Cytospins and Wright/Giemsa staining of whole kidney marrow cells isolated from wildtype fish (P) compared with FACS sorted GFP+ cells from two representative aggressive NK cell-like leukemias, showing large blastic cells with abundant basophilic, vacuolated cytoplasm (Q–R). (S–V) Embryonal rhabdomyosarcoma arising in *tp53*del/del fish micro-injected at the one-cell stage with linearized *rag2:kRAS^{G12D} + rag2:GFP*. Primary (S), transplanted (2°) (T), and serially transplanted ERMS (3°) (U,V). Whole animal bright-field images (A,D,F,J) and merged GFP-. Figure 2 continued on next page.
Figure 2 continued

fluorescence images (G,H,K,L,S–U). Hematoxylin and eosin stained sections of engrafted tumors (B,C,E, I, M,V). Scale bars are 5 mm in whole animal images and 100 μm for histology images.

DOI: https://doi.org/10.7554/eLife.37202.006
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**Figure 2—figure supplement 1.** Engraftment of tp53<sup>del/del</sup> tumors into CG1 recipient zebrafish. Engraftment was scored at >20 days post transplantation. i.p. intraperitoneal.

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Figure 3. Gene expression analysis of tp53<sup>del/del</sup> tumors. (A) Principal component analysis (PCA) of gene expression profiles from whole CG1 syngeneic fish, MPNSTs, a germ cell tumor, and FACS sorted GFP+ leukemia, angiosarcomas, and ERMS. All tumor samples were obtained following engraftment.

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Figure 3 continued on next page
Figure 3 continued

in CG1 syngeneic recipient fish. (B) Heat map of genes differentially expressed with respect to controls identifies molecularly defined tumor groups. (C) Upregulated genes identified within each tumor type are enriched for Molecular Signature Database (MSigDB) signatures consistent with the expected tissue of origin. (D) NK cell leukemias are enriched for gene signatures identified from normal NK and NKL cells in the kidney marrow and NK cells isolated from rag1<sup>−/−</sup>, tg(lck:GFP) transgenic fish (NK cells*). For each analysis, enrichment is shown for the top 30 lineage-restricted genes identified from single-cell transcriptional profiling of transgenic cells using SMARTseq2 (denoted by asterisks) or unsorted cells using InDrops single-cell RNA sequencing approaches. (E) Heat map highlighting NK lineage genes significantly upregulated in tp53<sup>del/del</sup> leukemias when compared to all other tumor types analyzed. [log<sub>2</sub>(fold-change)]. Angiosarcoma (AS) and germ cell tumor (GC).

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**Figure 3—figure supplement 1.** Zebrafish cancers share common gene expression with human tumors and confirmation of NK-cell lineage derivation for tp53<sup>del/del</sup> leukemias. (A–C) GSEA analysis comparing zebrafish tp53<sup>del/del</sup> tumors to human counterparts. (A) Human angiosarcoma (FDR q-value = 0.001), (B) human MPNST (FDR q-value = 0.0043526), and (C) human ERMS (FDR q-value = 0) gene sets are significantly enriched in the corresponding zebrafish tp53<sup>del/del</sup> tumors. (D) Heat map depicting differential gene expression in zebrafish tp53<sup>del/del</sup> leukemias compared to whole CG1 control animals. Gene-expression defines tp53<sup>del/del</sup> leukemias based on lineage signatures previously generated using InDrop and SMARTseq (asterisk) sequencing approaches (Tang et al., 2017). (E) Expression of the top 200 tp53<sup>del/del</sup> ANKL-like genes assessed in the SMARTseq dataset from Tang et al. (2017). HSC/progenitors were isolated as cd41:GFP<sup>low</sup> cells from transgenic zebrafish, T cells from tg(lck:GFP) transgenic zebrafish, NK cells from rag1<sup>-/-</sup>, tg(lck:GFP) transgenic zebrafish, myeloid cells from tg(mpx:EGFP) transgenic zebrafish, B cells from marrow-derived tg(rag2:GFP) transgenic zebrafish, and HSCs from tg(runx1<sup>+</sup>23:GFP) transgenic zebrafish. The tp53<sup>del/del</sup> leukemia gene expression signature was significantly enriched only in the NK cells (log2(TPM+1)≥2, p-value=0.015, one-sided binomial test).

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Figure 4. *tp53*del/del kRASG12D-induced ERMS have increased invasion and metastasis. (A–F) Whole animal fluorescent images of CG1-strain fish engrafted into the dorsolateral musculature with non-disseminated (A–C) and disseminated ERMS (D–F). Days post transplantation (dpt). White lines demarcate GFP+ tumor area. White arrowheads show site of injection and yellow arrowheads denote metastatic lesions. (G) H and E and (H) GFP immunohistological staining of fish engrafted with metastatic *tp53*del/del kRASG12D-induced ERMS. (I) Quantification of growth confined to site of injection (green bars) and compared with animals that exhibited local invasion or metastatic ERMS following tumor engraftment until fish were moribund. X-axis identifies 5 *tp53*wt/wt and 11 *tp53*del/del ERMS primary tumors that were transplanted into wild-type CG1 syngeneic host zebrafish. p=0.003, one-sided Fisher’s exact test. Scale bars denote 5 mm.

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