Cancers are composed of a heterogeneous mix of cells with varying differentiation, proliferation and tumorigenic properties (Heppner, 1984; Loebinger et al., 1984a; Al-Hajj et al., 2003). Indeed, in vivo studies have demonstrated that within a cancer population, only a small percentage of potential 'cancer stem cells' are able to initiate tumour development (Lapidot et al., 1994; Al-Hajj et al., 2003; Loebinger et al., 2008b) (Visvader and Lindeman, 2008). Conventional cancer treatments may eradicate the tumour bulk but spare these cells, which may explain why an initial tumour regression does not necessarily translate to an improved patient survival in many clinical trials for advanced cancers (Wicha et al., 2006).

The identification and destruction of these stem cells may therefore improve cancer treatment responses. Cell surface markers have been used in some cancers to produce a population of cells enriched with stem cell properties, for example, CD133 in the identification of human glioma (Singh et al., 2004) and colon cancer (Ricci-Vitiani et al., 2007) stem cells. However these markers appear specific to particular tumours and no marker has identified cancer stem cells across tumour types. Normal stem cell characteristics are often utilised to identify this population. This includes the ability to efflux nuclear dyes such as Hoechst 33342, which binds to DNA. The efflux of Hoechst is due to ABC transporters, in particular ABCG2/BCRP1 (Zhou et al., 2001). These Hoechst-effluxing cells were originally described in the bone marrow and termed 'side population' (SP) owing to their appearance on flow cytometry plots (Goodell et al., 1996).

They have since been shown in a variety of normal tissues, wherein they possess stem-like properties (Wu and Alman, 2008). They have also been identified in many cancers, including lung (Ho et al., 2007; Sung et al., 2008), breast (Engelmann et al., 2008), oesophageal (Huang et al., 2009), hepatocellular (Kamohara et al., 2008), glioma (Harris et al., 2008), renal (Addla et al., 2008) and squamous (Loebinger et al., 2008b) cancer cell lines, in addition to primary cancer cells (Barrett et al., 1995; Hirschmann-Jax et al., 2004; Szotek et al., 2006; Wu et al., 2007).

We, and others, have previously demonstrated that this Hoechst-effluxing, SP of cells within cancers have many stem-like properties, including the ability to re-populate both the SP and non-Hoechst effluxability non-side populations (non-SP) cell compartments, an increased ability to form colonies and generate complex spheroids in three-dimensional culture, a high telomerase activity and increased quiescence (Kondo et al., 2004; Ho et al., 2007; Addla et al., 2008; Loebinger et al., 2008b; Zhang et al., 2009). They have also been shown to express a number of stem-like genes (including OCT-4, SOX-2 and BMI-1) (Huang et al., 2009; Zhang et al., 2009), ABC transporter genes (including ABCG2) (Loebinger et al., 2008; Huang et al., 2009; Zhang et al., 2009), and genes involved in the Wnt (Haraguchi et al., 2006; Addla et al., 2008; Huang et al., 2009), Notch (Addla et al., 2008; Huang et al., 2009), PI3K/Akt pathways and cell cycle regulation (Zhou et al., 2007). Side population cells derived from both primary tumours (Wu et al., 2007) and cancer cell lines (Chiba et al., 2006; Ho et al., 2007; Loebinger et al., 2008b) also have an increased ability to initiate tumours compared with the majority of the tumour cells, when xenografted into immunodeficient mice. Furthermore, we, and others, have demonstrated that the SP cells are able to escape death...
by many chemotherapeutic agents, owing in part to their relative quiescence, in addition to increased ABC transporter expression which leads to the efflux of lipophilic chemotherapy agents such as mitoxantrone (Hirschmann-Jax et al, 2004; Loebinger et al, 2008b). This combination of resistance and tumour initiation makes it likely that these SP cells are central to tumour growth and recurrence, and stresses the importance of targeting these cells with future cancer therapies.

Mesenchymal stem cells (MSCs) have been used as delivery vehicles for targeted, antitumour therapies (Studeny et al, 2002; Loebinger et al, 2008a, c). These cells are derived from the adult bone marrow and have the ability to specifically home towards tumours throughout the body. In addition, they are immuno-privileged, enabling their use without rejection or immunosuppressive pre-conditioning. We, and others, have engineered MSCs to express TNF-related apoptosis-inducing ligand (TRAIL) (Loebinger et al, 2009; Szegedi et al, 2009; Grisendi et al, 2010). TRAIL is a protein which causes apoptosis and death of cancer cells, without harming normal cells, by binding to specific TRAIL receptors and leading to activation of the extrinsic apoptosis pathway (Wiley et al, 1995). In vivo studies have demonstrated that these cells are able to target multiple tumours and reduce primary and metastatic disease (Loebinger et al, 2009; Grisendi et al, 2010). The ability of this therapy to target and kill the putative cancer stem cells has not been determined. We hypothesised that MSC-delivered TRAIL therapy would target SP and non-SP cells equally.

**MATERIALS AND METHODS**

**Tissue culture**

Human adult MSCs were provided through the Tulane Centre for Gene Therapy, MSC cell distribution centre (New Orleans, LA, USA) and cultured in αMEM with 4 mM l-Glutamine, 50 U ml⁻¹ penicillin and 50 μg ml⁻¹ streptomycin, and 16% (v/v) fetal bovine serum. H357 cells were provided by Cancer Research UK, and cultured in a 1:3 mix of Hams F12 medium and Dulbecco’s modified Eagle’s medium (Janes and Watt, 2004). In addition to

**Figure 1** Squamous cancer and adenocarcinoma cancer cell lines contain a SP. (A, B) Representative flow cytometry plots demonstrating that H357 (A) and A549 cell lines (B) but not the MDAMB231 breast cancer cell line (C) contain a SP of cells that stain poorly with Hoechst. Cells were labelled with 7.5 mg ml⁻¹ Hoechst 33342 for 45 min. This population disappears with the ABC transporter inhibitor, reserpine (5 μM). The gates show the cells defined as SP and non-SP for the experiments.

**Figure 2** Mesenchymal stem cell TRAIL expression and killing effect compared with recombinant TRAIL. (A) ELISA demonstrating level of TRAIL produced in TRAIL transduced MSCs with and without addition of 10 μg ml⁻¹ doxycycline for 48 h. (B) Bar chart demonstrating the percentage of apoptosis in A549 cells with increasing levels of recombinant TRAIL (rT) 10–200 ng ml⁻¹ and doxycycline-induced TRAIL expression. Experiments were performed in triplicate. ***P<0.001.
fetal bovine serum, l-Glutamine and antibiotics, this medium was supplemented with 10−6 M cholera enterotoxin (ICN Pharmaceuticals Ltd., Oxon, UK), 0.5 g ml−1 hydrocortisone, 10 ng ml−1 epidermal growth factor and 5 μg ml−1 insulin (Janes et al., 2004). A549 and MDM2B231 cells were provided by Cancer Research UK and cultured in Dulbecco’s modified Eagle’s medium.

Production of TRAIL-transduced MSCs

Mesenchymal stem cells were transduced with membrane-bound TRAIL and green fluorescent protein, under the control of a Tetracycline-on promoter, using a lentivirus, as previously described (Loebinger et al., 2009). The transduced MSCs (MSCFLT) expressed TRAIL and green fluorescent protein only on addition of 10 μg ml−1 doxycycline (Loebinger et al., 2009). Human TRAIL expression was verified by ELISA (R&D Systems, Abingdon, UK) as per manufacturer’s instructions.

SP identification and sorting

To identify the SP, previously described protocols were followed (Loebinger et al., 2008b). Cancer cells were harvested at full confluence, re-suspended at 1 × 106 cells ml−1 in medium and labelled with 2.5, 5, or 7.5 μg ml−1 Hoechst 33342 for 45, 60, or 90 min at 37°C to determine the required incubation time. Dead cells were excluded with propidium iodide labelling. The multidrug transporter inhibitor, reserpine (5 μM) was used to demonstrate specificity of the SP. Analysis was performed on an LSR2 machine (Becton Dickenson, Oxford, UK). The optimal staining conditions were 5 μg ml−1 Hoechst 33342 for 45 min and 7.5 μg ml−1 Hoechst 33342 for 60 min for the H357 and A549 cells, respectively (Loebinger et al., 2008b). The SP and non-SP population (1 × 106 of each cell type) were sorted according to the gates in Figure 1 using a MoFlo High-Performance Cell Sorter (Dako, Glostrup, Denmark).

Co-culture

Cancer cells were stained with the fluorescent dye Dil (according to the manufacturer’s instructions, Invitrogen, Paisley, UK), before any cell sorting, and plated with MSCFLT cells (passage seven after transduction) in a 6-well plate (5 × 104 of each cell type). The following day doxycycline was added and left for 48 h. The early and late apoptosis of the cells in co-culture was assessed by Annexin V-based flow cytometry with DAPI or propidium iodide. Annexin V+/DAPI− (or PI−) cells were judged to be viable, Annexin V+/DAPI+ cells were considered to be undergoing early apoptosis, and Annexin V+/DAPI+ cells were considered late apoptotic (Loebinger et al., 2009). In subsequent assays, the cancer cells were pre-treated with 10 ng ml−1 of mitoxantrone before the addition of 5 × 104 MSCs (passage 9 and 10) or apoptosis induced by MSCFLT was compared with recombinant TRAIL (using manufacturer’s guideline doses) for 48 h.

Colony formation

In all, 200 Dil-stained and then freshly sorted, SP or non-SP H357 cells were added to a 6-well plate. The following day, 5 × 104 MSCFLT cells, treated with mitomycin C to prevent their continued proliferation were added to the plates and the TRAIL transgene either activated or not with doxycycline. After 14 days of co-culture, colonies were washed, fixed using 3% PFA, respectively (Loebinger et al., 2008b). The SP and non-SP population (1 × 106 of each cell type) were sorted according to the gates in Figure 1 using a MoFlo High-Performance Cell Sorter (Dako, Glostrup, Denmark).

Co-culture

Cancer cells were stained with the fluorescent dye Dil (according to the manufacturer’s instructions, Invitrogen, Paisley, UK), before any cell sorting, and plated with MSCFLT cells (passage seven after transduction) in a 6-well plate (5 × 104 of each cell type). The following day doxycycline was added and left for 48 h. The early and late apoptosis of the cells in co-culture was assessed by Annexin V-based flow cytometry with DAPI or propidium iodide. Annexin V+/DAPI− (or PI−) cells were judged to be viable, Annexin V+/DAPI+ cells were considered to be undergoing early apoptosis, and Annexin V+/DAPI+ cells were considered late apoptotic (Loebinger et al., 2009). In subsequent assays, the cancer cells were pre-treated with 10 ng ml−1 of mitoxantrone before the addition of 5 × 104 MSCs (passage 9 and 10) or apoptosis induced by MSCFLT was compared with recombinant TRAIL (using manufacturer’s guideline doses) for 48 h.

Colony formation

In all, 200 Dil-stained and then freshly sorted, SP or non-SP H357 cells were added to a 6-well plate. The following day, 5 × 104 MSCFLT cells, treated with mitomycin C to prevent their continued proliferation were added to the plates and the TRAIL transgene either activated or not with doxycycline. After 14 days of co-culture, colonies were washed, fixed using 3% PFA, respectively (Loebinger et al., 2008b). The SP and non-SP population (1 × 106 of each cell type) were sorted according to the gates in Figure 1 using a MoFlo High-Performance Cell Sorter (Dako, Glostrup, Denmark).

Co-culture

Cancer cells were stained with the fluorescent dye Dil (according to the manufacturer’s instructions, Invitrogen, Paisley, UK), before any cell sorting, and plated with MSCFLT cells (passage seven after transduction) in a 6-well plate (5 × 104 of each cell type). The following day doxycycline was added and left for 48 h. The early and late apoptosis of the cells in co-culture was assessed by Annexin V-based flow cytometry with DAPI or propidium iodide. Annexin V+/DAPI− (or PI−) cells were judged to be viable, Annexin V+/DAPI+ cells were considered to be undergoing early apoptosis, and Annexin V+/DAPI+ cells were considered late apoptotic (Loebinger et al., 2009). In subsequent assays, the cancer cells were pre-treated with 10 ng ml−1 of mitoxantrone before the addition of 5 × 104 MSCs (passage 9 and 10) or apoptosis induced by MSCFLT was compared with recombinant TRAIL (using manufacturer’s guideline doses) for 48 h.

Colony formation

In all, 200 Dil-stained and then freshly sorted, SP or non-SP H357 cells were added to a 6-well plate. The following day, 5 × 104 MSCFLT cells, treated with mitomycin C to prevent their continued proliferation were added to the plates and the TRAIL transgene either activated or not with doxycycline. After 14 days of co-culture, colonies were washed, fixed using 3% PFA,
and stained with Rhodanile Blue overnight. Colonies were counted using an Olympus CK2 inverted phase-contrast light microscope (Olympus, Essex, UK). A large colony was defined as greater than 32 cells per colony and abortive colonies were defined as colonies that contained fewer than 32 cells (Loebinger et al., 2008b).

RESULTS

Squamous and lung cancer cell lines contain an ABC transporter SP

Many cancer cell lines and primary cells contain a SP. In order to identify whether squamous cell, lung and breast carcinomas contain a similar subpopulation of drug-resistant SP cells, confluent H357 (squamous), A549 (lung) and MDA-MB-231 (breast) cancer cell lines were incubated with Hoechst 33342 dye and analysed by flow cytometry. A characteristic SP fraction was detected in the H357 and A549 cell lines, but not in the MDA-MB-231 cells, which is consistent with a previous report (Engelmann et al., 2008). The squamous and lung SPs were both reserpine sensitive, indicating their dependence on ABC-type transporter activity (Figures 1A–C).

The SP cells can be killed by TRAIL-expressing MSCs

TRAIL-expressing MSCs have previously been shown to have the ability to cause cancer cell death and decrease tumour and metastasis development in vivo (Loebinger et al., 2009). In these experiments, the TRAIL lentivirus is conditionally activated with the addition of doxycycline, and carries green fluorescent protein to enable the monitoring of gene activation. We initially confirmed the production of TRAIL after activation of the transgene. MSCFLT cells were cultured with or without doxycycline for 48 h and cells harvested for protein measurement by ELISA. Doxycycline treatment led to 660 vs 2.92 pg ml$^{-1}$ ($P = 0.001$) without treatment (Figure 2A). In co-culture experiments using H357 cells, we confirmed sensitivity of unsorted populations of these cells to doxycycline treated MSCFLT cells and found H357 cells more sensitive to MSCFLT cells than high doses of recombinant protein (Figure 2B) as previously seen by our laboratory (Loebinger et al., 2009).

The cancer stem cell hypothesis suggests that their destruction is crucial for a cancer therapy. Having isolated a population enriched for possible cancer stem cells (SP) in both squamous and lung cancer cell lines, their susceptibility to the doxycycline-controlled, TRAIL-expressing MSC therapy was tested in co-culture experiments. SP and non-SP cells were freshly flow-sorted from Dil-stained H357 and A549 cells (Figure 1) and immediately co-cultured with the MSCFLT cells. The death and apoptosis of the cancer cells in co-culture was assessed by Annexin V flow cytometry. There was a significant increase in apoptosis of both the SP and non-SP subgroups of A549 cells with the use of doxycycline and activation of the TRAIL transgene (SP: 2.7% increased to 27.7% ± 1.9%, non-SP: 4.4% ± 1.5% increased to 30.4% ± 1.6%) (Figure 3A–C) and H357 cells (SP: 13.4% ± 1.4% increased to 68.1% ± 5.5%, non-SP: 16.1% ± 1.4% increased to 60.9% ± 2.5%) (Figure 3D) (both $P < 0.001$, Anova).

The susceptibility of the cancer cell subpopulations to TRAIL-expressing MSCs was further assessed with colony forming assays. In all, 200 Dil-stained and then freshly sorted, SP or non-SP H357 cells were added to a 6-well plate. The following day, $5 \times 10^4$ MSCFLT cells (treated with mitomycin C to prevent their further

Figure 4  TRAIL-expressing MSCs reduce the clonogenic potential of H357 SP and non-SP cells and produce additional SP cancer cell killing to mitoxantrone treatment. (A, B) In all, 200 Dil-labelled, SP (A) or non-SP (B) H357 cells were plated for colony forming assays before the addition of $5 \times 10^4$ TRAIL-expressing MSCs (MSCFLT). (C) Quantification of large colony numbers from (A, B) demonstrates a reduction in large colonies with doxycycline (dox)-induced TRAIL expression in both SP and non-SP cells compared with the co-cultures without dox (ND). Furthermore, SP cells produced more colonies than non-SP cells. (D) Phase-contrast and (E) fluorescent microscopy demonstrate the green fluorescent protein (green) from the doxycycline (dox)-activated MSCFLT surrounding the Dil-labelled (red) H357 colonies. (F) SP cells were exposed to mitoxantrone (Mitox) and then co-cultured with MSCFLT cells. Bar chart represents triplicate flow cytometry experiments and demonstrates a further increase in death and apoptosis of the SP cells with the addition of doxycycline and activation of MSCFLT TRAIL expression. ***$P < 0.001$, **$P < 0.01$, ns, non-significant. Scale bars represent 25 μm.
The addition of TRAIL-expressing MSCs to mitoxantrone treatment causes further cancer cell killing

The Dil-labelled, SP H357 cells were treated with 10 ng ml$^{-1}$ mitoxantrone for 3 days followed by co-culture with MSCFLT cells. The Annexin V flow cytometry assay was used to determine the early and late apoptotic SP cancer cells. There was a significant increase in apoptotic cells with the addition of doxycycline and activation of the TRAIL transgene (56.3 ± 10.0% with doxycycline, compared with 33.9 ± 2.4% without doxycycline) (P<0.01, Anova) (Figure 4F). This suggests a further cancer killing effect of the TRAIL-expressing MSCs above and beyond the mitoxantrone chemotherapeutic agent. Indeed, when TRAIL was not expressed with doxycycline, the use of mitoxantrone alone did not significantly increase the early and late apoptotic cancer cells, consistent with a degree of mitoxantrone chemoresistance of the SP cells as discussed above (33.9 ± 2.4% with mitoxantrone compared with 24.56 ± 4.2% without mitoxantrone) (P>0.05, Anova) (Figure 4F).

**DISCUSSION**

One feature of the SP, or subgroup of cells enriched for stem cells, is their resistance to common oncological treatments (Loeberger et al, 2009). These studies have demonstrated that this subpopulation has some resistance to chemotherapy agents such as mitoxantrone. This subgroup of cells has also been shown to be more capable of tumour initiation in subcutaneous models (Hirschmann-Jax et al, 2004; Loeberger et al, 2008b). The combination of increased treatment resistance and ability to re-populate tumours suggests new treatments should target these cell populations.

**ACKNOWLEDGEMENTS**

Funding: MRL is a Medical Research Council (MRC) UK Clinical Training Fellow. EKS is a Mick Knighton Mesothelioma Fund British Lung Foundation MRC UK Clinical Training Fellow. SMJ is a Wellcome Trust Senior Research Fellow in Clinical Science. This work was partly undertaken at UCLH/UCL, which received a proportion of funding from the Department of Health’s NIHR Biomedical Research Centre funding scheme.

**Conflict of interest**

The authors declare no conflict of interest.

**REFERENCES**

Addla SK, Brown MD, Hart CA, Ramani VA, Clarke NW (2008) Characterization of the Hoechst 33342 side population from normal and malignant human epithelial cells. *Am J Physiol Renal Physiol* 295: F680–F687

Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF (2003) Prospective identification of tumourigenic breast cancer cells. *Proc Natl Acad Sci USA* 100: 3983–3988

Barrett P, Hobbs RC, Coates PJ, Risdon RA, Wright NA, Hall PA (1995) Endocrine cells of the human gastrointestinal tract have no proliferative capacity. *Histochem J* 27: 482–486

Chiba T, Kita K, Zheng YW, Yokosuka O, Saisho H, Iwama A, Nakauchi H, Barrett P, Hobbs RC, Coates PJ, Risdon RA, Wright NA, Hall PA (1995) Endocrine cells of the human gastrointestinal tract have no proliferative capacity. *Histochem J* 27: 482–486

Chiba T, Kita K, Zheng YW, Yokosuka O, Saisho H, Iwama A, Nakauchi H, Taniguchi H (2006) Side population purified from hepatocellular carcinoma cells harbors cancer stem cell-like properties. *Hepatology* 44: 240–251

Engelmann K, Shen H, Finn OJ (2008) MCF7 side population cells with characteristics of cancer stem/progenitor cells express the tumour antigen MUC1. *Cancer Res* 68: 2419–2426

Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC (1996) Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. *J Exp Med* 183: 1797–1806

Grisendi G, Bussolari R, Cafarelli L, Petak I, Rasini V, Veronesi E, De Santis G, Spano C, Tagliuzzucchi M, Barti-Juhasz H, Scarabelli L, Bambi F, Frassoldati A, Rossi G, Casali C, Morandi U, Horwitz EM, Paolucci P, Conte P, Dominici M (2010) Adipose-derived mesenchymal stem cells as stable source of tumour necrosis factor-related apoptosis-inducing ligand delivery for cancer therapy. *Cancer Res* 70: 3718–3729

Haraguchi N, Utsunomiya T, Inoue H, Tanaka F, Mimori K, Barnard GF, Mori M (2006) Characterization of a side population of cancer cells from human gastrointestinal tumours. *Stem Cells* 24: 506–513

Harris MA, Yang H, Low BE, Mukherje J, Bronson RT, Shultz LD, Israel MA, Yun K (2008) Cancer stem cells are enriched in the side population cells in a mouse model of glioma. *Cancer Res* 68: 10051–10059

Heppner GH (1984) Tumour heterogeneity. *Cancer Res* 44: 2259–2265

Hirschmann-Jax C, Foster AE, Wulf GG, Nuchtern JG, Jax TW, Gobel U, Goodell MA, Brenner MK (2004) A distinct ‘side population’ of cells with high drug efflux capacity in human tumour cells. *Proc Natl Acad Sci USA* 101: 14228–14233

Ho MM, Ng AV, Lam S, Hung JY (2007) Side population in human acute leukaemia cells and tumours is enriched with stem-like acute leukaemia cells. *Cancer Res* 67: 4827–4833

Huang D, Gao Q, Guo L, Zhang C, Jiang W, Li H, Wang J, Han X, Shi Y, Lu SH (2009) Isolation and identification of cancer stem-like cells in esophageal carcinoma cell lines. *Stem Cells Dev* 18: 465–473

**growth** were added to the plates and the TRAIL transgene either activated or not with doxycycline. As expected from previous work (Loeberger et al, 2008b), colony formation was greater in the SP subgroup compared with the non-SP cells (60.0 ± 1.7 large colonies in the SP cells compared with 34.0 ± 7.8 large colonies in the non-SP cells) (P<0.001, two-way Anova). Colony formation was significantly inhibited in both cell subgroups with the doxycycline-induced activation of the TRAIL transgene of the MSCs (SP: 60 ± 1.7 large colonies reduced to 14.7 ± 4.2 large colonies with TRAIL expression, non-SP: 34.0 ± 7.8 large colonies reduced to 8.0 ± 1.7 large colonies with TRAIL expression) (both P<0.001, two-way Anova) (Figure 4A–E).
Janes SM, Ofstad TA, Campbell DH, Watt FM, Prowse DM (2004) Transient activation of FOXN1 in keratinocytes induces a transcriptional programme that promotes terminal differentiation: contrasting roles of FOXN1 and Akt. J Cell Sci 117: 4157–4168

Janes SM, Watt FM (2004) Switch from \(\alpha v\beta 5\) to \(\alpha v\beta 6\) integrin expression protects squamous cell carcinomas from anoikis. J Cell Biol 166: 419–431

Kamohara Y, Haraguchi N, Mimori K, Tanaka F, Inoue H, Mori M, Kanematsu T (2008) The search for cancer stem cells in hepaticocellular carcinoma. Surgery 144: 119–124

Kondo T, Setoguchi T, Taga T (2004) Persistence of a small subpopulation of cancer stem-like cells in the C6 glioma cell line. Proc Natl Acad Sci USA 101: 781–786

Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, Minden M, Paterson B, Caligiuri MA, Dick JE (1994) A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature 367: 645–648

Loebinger MR, Aguilar S, Janes SM (2008a) Therapeutic potential of stem cells in lung disease: progress and pitfalls. Clin Sci (Lond) 114: 99–108

Loebinger MR, Eddaoudi A, Davies D, Janes SM (2009) Mesenchymal stem cell delivery of TRAIL can eliminate metastatic cancer. Cancer Res 69: 4134–4142

Loebinger MR, Giangreco A, Groot KR, Prichard L, Allen K, Simpson C, De Maria R (2007) Identification and expansion of human colon-cancer-initiating cells. Nature 445: 111–115

Masportas LS, Kasmiieh R, Wakimoto H, Hingtgen S, van de Water JA, Mohapatra G, Figueiredo JL, Martuza RL, Weissleder R, Shah K (2009) Assessment of therapeutic efficacy and fate of engineered human mesenchymal stem cells for cancer therapy. Proc Natl Acad Sci USA 106: 4822–4827

Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB (2004) Identification of human brain tumour initiating cells. Nature 432: 396–401

Studenyn M, Marini FC, Champlin RE, Zompetta C, Filider IJ, Andreeff M (2002) Bone marrow-derived mesenchymal stem cells as vehicles for interferon-beta delivery into tumours. Cancer Res 62: 3603–3608

Sung JM, Cho HJ, Yi H, Lee CH, Kim HS, Kim DK, Abd El-Aty AM, Kim JS, Landowski CP, Hediger MA, Shin HC (2008) Characterization of a stem cell population in lung cancer A549 cells. Biochem Biophys Res Commun 371: 163–167

Sussmann RT, Ricci MS, Hart LS, Sun SY, El-Deiry WS (2007) Chemoresistant side-population of colon cancer cells has a higher sensitivity to TRAIL than the non-SP, a higher expression of c-Myc and TRAIL-receptor DR4. Cancer Biol Ther 6: 1490–1495

Szegedi E, O’Reilly A, Davy V, Vawda R, Taylor DL, Murphy M, Samali A, Mehmet H (2009) Stem cells are resistant to TRAIL receptor-mediated apoptosis. J Cell Mol Med 13: 4409–4414

Sztok PP, Pieretti-Vannarcke R, Masiakos PT, Dinulescu DM, Connolly D, Foster R, Dombkowski D, Preffer F, Maclaughlin DT, Donahoe PK (2006) Ovarian cancer side population defines cells with stem cell-like characteristics and Mullerian Inhibiting Substance responsiveness. Proc Natl Acad Sci USA 103: 11154–11159

Visvader JE, Lindeman GJ (2008) Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. Nat Rev Cancer 8: 755–768

Wicha MS, Liu S, Dontu G (2006) Cancer stem cells: an old idea—a paradigm shift. Cancer Res 66: 1883–1890; discussion 1895–6

Wiley SR, Schooley K, Smolik PJ, Din WS, Huang CP, Nicholl JK, Sutherland GR, Smith TD, Rauch C, Smith CA, Goodwin RG (1995) Identification and characterization of a new member of the TNF family that induces apoptosis. Immunity 3: 673–682

Wu C, Alman BA (2008) Side population cells in human cancers. Cancer Lett 268: 1–9

Wu C, Wei Q, Uotomo V, Nadesan P, Whetstone H, Kandel R, Wunder JS, Alman BA (2007) Side population cells isolated from mesenchymal neoplasms have tumour initiating potential. Cancer Res 67: 8216–8222

Zhang P, Zhang Y, Mao L, Zhang Z, Chen W (2008) Side population in oral squamous cell carcinoma possesses tumour stem cell phenotypes. Cancer Lett 277: 227–234

Zhang X, Komaki R, Wang L, Fang B, Chang JY (2008) Treatment of radioresistant stem-like esophageal cancer cells by an apoptotic gene-armed, telomerase-specific oncolytic adenovirus. Clin Cancer Res 14: 2813–2823

Zhou J, Wu,kuhlke J, Zhang H, Gu P, Yang Y, Deng J, Margolick JB, Liotta LA, Petricoin III E, Zhang Y (2007) Activation of the PTEN/mTOR/STAT3 pathway in breast cancer stem-like cells is required for viability and maintenance. Proc Natl Acad Sci USA 104: 16158–16163

Zhou S, Schuetz JD, Bunting KD, Colapietro AM, Sampath J, Morris JJ, Lagutina I, Grosveld GC, Osawa M, Nakauchi H, Sorrentino BP (2001) The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. Nat Med 7: 1028–1034