Journal Reviews

Neuro-Oncology

DNA fingerprinting in glioma cell lines

Brady P, Diserens AC, Castella V, Kalt S, Heinimann K, Hamou MF, Delorenzi M, Hegi M. DNA fingerprinting of glioma cell lines and considerations on similarity measurements. Neuro-Oncology 2012;14(6):701-711.

Glioma cell lines form a major part of novel in vivo and in vitro studies in brain tumour research. However, cross contamination of cell lines can lead to erroneous results. As a result, cell line validation is now a standard part of many journal and grant body submission requirements. In this study, the authors reference 16 marker short tandem repeat (STR) DNA fingerprints for 39 of the commonest glioma cell lines, 3 of which were glioma-derived sphere lines, 24 of them being established in the authors' laboratory. Fingerprints were compared amongst the cell lines and with a publically available database of 9 STRs (DSMZ Database). Cell lines with a similar score >0.8 were compared, which showed 3 misidentified cell lines, of which 2 were characterised as sub-lines of other glioma cell lines within the same laboratory (LN319 sub-line of LN992 and LN443 sub-line of LN444), and SF767 which was identical to ME180 – a squamous cell carcinoma line. A similarity score of 0.8 is often used as a cut-off indicating cross-contamination. When 9 STRs were randomly rearranged, 1 profile had a score of >0.8, 8 profiles had a score of >0.7, and when all markers were used, no pairs were >0.8. The authors conclude that the 9 STR marker-set did not reliably distinguish between the true origin of cell lines when compared to 16 STR panels when a cut-off value of 0.8 was used.

Reviewer's opinion: This study highlights the importance of validation of cell lines used in research and suggests a more robust panel of DNA fingerprints for this purpose. In the future, journals may request more detailed information regarding cell line origins, validation and procedures when papers are submitted for publication. – SB

Tumour-initiating cells in human glioma

Clement-Schatlo V, Marino D, Burkhardt K, Teta P, Leyvraz F, Schatlo B, Frank S, Schaller K, Castella V, Radovanovic I. Quantification, self-renewal and genetic tracing of FL1+ tumour initiating cells in a large cohort of human gliomas. Neuro-Oncology 2012;14(6):720-35.

A subset of tumour-initiating cells (GICs) with stem cell-like features may be responsible for tumour growth and recurrence in glioma. Identification of these cells with stem markers, such as CD133, proved inconsistent. In a previous study, the authors had identified this tumour cell subpopulation using morphological features (large cells with high nuclear/cytoplasmic ratio) and the presence of autofluorescence emission (FL1+). In this study, they have validated these results in a larger cohort; 74 primary brain tumour samples and tumours from a mouse model (GFAP-V12HA-ras B8) were cultured in stem cell media and assessed for the percentage of FL1+ cells and capability for self-renewal. Low grade tumours had low numbers of FL1+ cells and a low capacity for long-term culture (<5 passages). In contrast, high grade tumours showed higher numbers of FLV1+ cells, with those derived from Grade IV tumours being best for long-term culture (11/37 cultures >10 passages) and acquiring high levels of autofluorescence. FLV1+ cells were identified in control samples derived from epilepsy surgery; however, these did not acquire high levels of autofluoresence and showed a low propensity for self-propagation, nor were they tumourigenic when injected into the brains of nude mice. The DNA fingerprints of these cultures assessed using microsatellites showed genetic stablility in all but one of the specimens.

Reviewer's opinion: A novel method for the identification of glioma initiating cells in vitro is advanced, and adds to the literature of methods identifying subgroups of glioma cells within tumour populations. Further work on the phenotypic characterisation of glioma initiating cells within in vivo models and human tissue will be invaluable. – SB

Pseudoprogression, tumour recurrence and MRI

Hu L, Eschbacher JM, Heiserman J, Dueck A, Shapiro W et al. Re-evaluating the imaging definition of tumour progression: perfusion MRI quantifies recurrent glioblastoma tumour fraction, pseudoprogression and radiation necrosis to predict survival. Neuro-Oncology 2012;14(7):919-30.

Contrast enhanced MRI is used to measure treatment response in glioblastoma (GBM) and define progression free survival (PFS). However, pseudoprogression and radiation necrosis can radiologically mimic tumour regrowth and spread. Radiation changes are often evident along with tumour recurrence. Previous studies suggested that the tumour burden within these radiation changes may predict survival in patients with recurrent tumours. In this study, the authors recruited 25 patients with recurrent GBM (previously treated with combined chemoradiotherapy) who underwent image-guided re-resection of an enlarging lesion. 64% (16/25) developed lesions within 6 months of treatment and 36% (9/25) developed lesions over 6 months after treatment (range 8-53 months). Multiple perfusion MRI (pMRI)-based metrics were recorded for pre-operative contrast enhancing lesions and correlated with histological tumour fraction and overall survival. The histological tumour fraction correlated most strongly with pMRI-FTB ($r=0.82$; $P<0.0001$), and was the sole imaging factor that correlated with OS ($P<0.2$).

Reviewer's opinion: This study assesses the use of perfusion MRI in estimating tumour fraction within enlarging mass lesions post treatment for GBM. This is a difficult area as GBMs are typically infiltrative, and therefore the tumour burden within a resection specimen or debulking may not be representative of the entire lesion. Regardless, experimental MRI techniques are likely to become an important tool in the assessment of treatment response in the future. Further studies correlating radiological and pathological findings are essential. – SB

ABCB1 and hypoxia in glioblastoma

Chou CW, Wang CC, Wu CP, Lin YJ, Chun Lee Y, Cheng YW, Hseih CH. Tumour cycling hypoxia induces chemoresistance in glioblastoma multiforme by upregulating the expression and function of ABCB1. Neuro-Oncology 2012;14(10):1227-38.

Chemoresistance is an inherent feature of glioblastoma (GBM) that contributes to its poor prognosis. Many factors may be responsible for this phenomenon, including tumour hypoxia. Cycling hypoxia as a result of reduced tumour blood flow may limit chemotherapy responsiveness, although the mechanisms are unknown. ABCB1, an efflux pump, is expressed by GBM cells and can be regulated by hypoxia inducible factor 1 (HIF1⇨). These authors investigated the effect of cycling hypoxia on chemoresistance and ABCB1 functionboth in vitro and in vivo. There was higher HIF1⇨ and ABCB1 expression (protein and mRNA) in cycling hypoxic tumour cells (U87 and GBM8401) compared to chronic hypoxic cells and normoxic cells. Following transfection of U87 cells with a lentiviral vector containing an ABCB1 promoter-driven luciferase reporter, there was a significant increase in transcriptional activation of ABCB1 in the cycling hypoxia cells. In GBM, cycling hypoxia results in ABCB1 induction via upregulation of HIF1⇨. Using MTT assays, cycling hypoxia also resulted in increased chemoresistance to BCNU and doxorubicin compared to normoxic controls. ABCB1 overexpression also resulted in increased chemoresistance, suggesting its importance in the controlling pathway. In xenograft models, immunofluorescence

for ABCB1, HIF1 and Hoeschst 3342 (a perfusion marker) showed strong co-localisation in perfused areas of the tumour. Again, xenograft-derived cycling hypoxia tumour cells had higher ACBC1 expression and chemoresistance compared to chronic hypoxic and normoxic controls.

Reviewer's opinion: This study shows that the microenvironment plays an important role in the maintenance of tumour growth and response to therapy. Hypoxia is responsible for part of the chemoresistance commonly found in these tumours and may be a potential synergistic target for future multimodal directed therapies. – SB

Clinical Colorectal Cancer

Long-term results of 2 adjuvant trials reveal differences in chemosensitivity and the pattern of metastases between colon cancer and rectal cancer

Kornmann M, Staib L, Wiegel T et al. Clinical Colorectal Cancer 2013;12(1):54-61.

Since the intergroup studies a decade ago it has been accepted that combining folinic acid (FA) with 5-FU has significant survival benefits in adjuvant treatment for colon cancer. No such benefit has been established in rectal cancer, the treatment of which has undergone a greater paradigm shift with the introduction of both neoadjuvant radiotherapy and total mesorectal excision (TME) surgery. Both techniques have resulted in reduced rates of local recurrence, but neither influences survival, which is related to the development of distant metastases in 40% of patients. This paper reports a comparison of identically-designed randomised controlled trials of adjuvant chemotherapy using combinations of 5-FU, FA and interferon-alpha in locally advanced colon (FOGT1) and rectal (FOGT2) cancers. Both trials have previously reported that interferon-alpha increased toxicity without survival benefit and, in keeping with previous data, the addition of FA did not improve 5-year overall survival in rectal cancer, either for stage III (node-positive) tumours or stage II and II combined, although a non-significant trend towards reduced local recurrence and improved survival was seen in node-negative rectal cancer. Since all rectal cancer patients in the trial received 50Gy of postoperative radiotherapy, the authors postulate that FA acts as an additional radiosensitiser, without potentiating the effects of chemotherapy on distant metastases, i.e. adenocarcinoma of the rectum and colon may not be the same disease.

Reviewer's opinion: Given its good toxicity profile, there seems to be no reason to withhold folinic acid from 5-FU in the adjuvant treatment of node-negative rectal tumours; however the benefit in locally advanced node-positive rectal cancer is negligible. – JRN

Clinical Breast Cancer

Axillary nodal irradiation: is it a feasible alternative to axillary dissection in clinically node negative, sentinel node positive breast cancers?

Outcomes of clinically node-negative breast cancer without axillary dissection: can preserved axilla be safely treated with radiation after a positive sentinel node biopsy? Sanuki N, Takeda A, Amemiya A, Ofuchi T, Ono M, Ogata H, Yamagami R, Hatayama J, Eriguchi T, Kunieda E.Clinical Breast Cancer 2013;Feb; 13(1):69-76.

This study analysed the role of axillary nodal irradiation as a feasible alternative to axillary dissection for a safe control of early stage breast cancer, including those patients with a positive sentinel lymph node biopsy. It also compared and analysed the toxicity profiles of nodal and regional irradiation with clinically negative and sentinel node positive patient groups. Over 2100 Japanese patients from 3 institutions were studied over a 23-year period. All patients had cT1-T2N0MO disease and were classified into 3 groups of nx (n=1548) without any axillary surgery; the sn- group (n=518) with negative SLNB; and sn+ (n=104) with positive SLNB. The median follow-up times for were 88 (nx), 56 (sn-) and 55 (sn+) months, respectively. Ninety-eight percent of the sn-group received only tangent irradiation to axilla and 100 and 83% of the sn+ and nx group, respectively, received additional regional nodal irradiation. The 5-year cumulative incidences of axillary failure and regional nodal failure for nx, sn- and sn+ were 34, 3, and 0 (2.7%, 0.7%, and 0%; P = 0.02, log-rank test) and 57, 4, and 0 (4.4, 1%, and 0; P =0.04), respectively. Overall survival rates in 5 years for nx, sn- and sn+ were 96.4%, 98.9%, and 97.6% (P =0.03), respectively. Symptomatic but transient radiation pneumonitis developed in 31, 16, and 6 (2.0, 3.1, and 5.7%). Mild arm oedema was observed in 1, 4, and 0 (0.06, 0.8, and 0%) in the nx, sn-, sn+ groups, respectively. In conclusion, treatment without axillary dissection showed excellent outcome with negligible toxicity for patients with clinically node negative, including those with a positive SLNB. Regional nodal irradiation after a positive SLNB is a reasonable alternative to axillary dissection.

Reviewer's opinion: Follow-up data is reported on treating clinically node negative and sentinels node biopsy patients with nodal and regional irradiation therapy. The findings highlight excellent efficacy of irradiation in controlling axillary and regional disease in nx and sn+ groups at ~5 year follow-up. Compared to sn- and sn+ groups, the nx group showed worse regional control. This difference in responses between the groups is thought to be due to the use of different chemotherapy regimen in the nx group. The usefulness of treating regional lymph nodes (SCF) with positive SLNB using nomograms to predict the residual axillary burden was also highlighted. Lastly, the low irradiation toxicity profiles compared to ALNC in isolation or ALNC with regional irradiation augur well for employing these approaches in similar studies and assess long-term data. - TH