REVIEW

Potential use of the comet assay in the clinical management of cancer

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The comet assay has been widely used to measure a range of cellular responses to DNA damage and has found applications in genotoxicity studies, bio-monitoring, eco-logical testing and in the study of human disease. This review discusses how the comet assay has been applied to the study of DNA damage and repair associated with cancer. The potential of the assay as a tool for predicting an individual's tumour sensitivity to radiation and to various chemotherapeutic drugs is examined, as well as outlining the usefulness of the assay in assessing oxidative stress within tumours. In addition, we review the use of the comet assay in investigations of the DNA-damaging effect of anti-neoplastic drugs and radiation used during cancer therapy. The advantages and limitations of the comet assay in carrying out all these studies are outlined, and the suitability of the comet assay for use in the clinical management of cancer is discussed.

Introduction

The comet assay is now widely accepted as a standard method for assessing DNA damage in individual cells. It has been used in a broad variety of applications including human bio-monitoring, genotoxicology, ecological monitoring and as a tool to investigate DNA damage and repair in different cell types in response to a range of DNA-damaging agents (1). In addition, many studies have successfully demonstrated how the comet assay can be applied to the analysis of cells derived from a variety of different human tissues, lending considerable weight to the suggestion that this assay could benefit many areas of clinical investigation by providing valuable informa-tion about the intrinsic DNA characteristics of individual cells and their responses to various external factors, such as radiation, chemicals and drugs. This type of information would prove particularly relevant in the diagnosis, prognosis and treatment of cancer. Readily available accurate and informative data are fundamental to the successful management of tumours with the ultimate aim of achieving a positive outcome for the patient.

From a practical point of view, the comet assay would be eminently suitable for use in a clinical setting since it is a relatively simple and inexpensive technique, which requires only a few cells and results can be obtained within a matter of hours. However, despite these advantages, it has not been widely employed as a standard analytical tool within clinical laboratories, partly because there is still some doubt about its reliability, reproducibility and validity. Hence, in this paper, we focus on the applications of the comet assay in the study of cancer, reviewing studies that have investigated DNA damage and repair, mutagen sensitivity and biomarker potential in human tumour cells to help determine whether the comet assay really does have promise for clinical testing for cancer and what aspects of the assay need to be addressed in order to provide convincing reassurances about its potential worth and usefulness.

Use of the comet assay in cancer studies

DNA damage and defective DNA repair are the underlying molecular events driving the initiation and progression of cancer. It is therefore not surprising that many studies have used the comet assay to investigate DNA damage and repair characteristics in a wide range of tumour cells in response to a variety of DNA-damaging agents. These studies include both investigations on human tumour cell lines and on tumour cells extracted from cancer patients. Table I demonstrates the range of cancers and end points that have been assessed to date in cells extracted from clinical biopsy material.

Many of these studies show that results generated by the comet assay provide important information about the nature of the particular cancer, which could be used by oncologists to inform them as to the best possible course of intervention. As discussed below, these studies, and others, provide a practical solution to some of the problems encountered in the treatment of cancer.

Prediction of tumour radiosensitivity

The ability to predict the radiosensitivity of individual tumours has long been considered the 'holy grail' of radiation biology (18). A number of different factors will be considered in deciding upon a therapeutic radiation regimen for a cancer patient, but unfortunately there is still no definitive way of predicting whether the tumour will respond to radiotherapy. Tumour response to treatment will inevitably vary due to a range of factors including the type of tumour being treated, as well as the specific genetic make-up of the individual patient (19). At the cellular level, whether or not a tumour cell will die depends on factors such as the type of radiation used, cell-cycle position, oxygen level within the cell and the expression of various oncogenes and growth factors (20). With so many variables capable of exerting an influence on response to radiotherapy, it is not surprising that a wide variability in patient response exists. Therefore, it seems clear that a clinical test for accurately predicting tumour response to radiation on an individual basis would be a valuable asset in the cancer clinic.

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Table I. Comet assay studies examining cells extracted from human tumour biopsies[a]

Tumour type	Cells examined	Result	Reference
Oesophageal	Mucosal biopsies from Barrett's oesophagus and oesophageal adenocarcinomas	DNA damage significantly increased in cancer group compared to controls	(2)
Colorectal	Biopsies from colorectal adenocarcinomas	DNA damage significantly increased in cancer group compared to controls	(3)
Colon	Epithelial cells isolated from colon biopsies	BrdU comet assay detected defects in replication in tumour and pre-malignant colonic cells	(4)
Breast	Tumour cells obtained by fine-needle aspirate	DNA damage detected, related to patient metabolism of ifosfamide and doxorubicin	(5)
Breast	Tumour cells obtained by fine-needle aspirate immediately following radiotherapy	Hypoxic tumour cells successfully detected following radiation induced damage	(6) and (7)
Lung	Lymphoid cells from lung cancer patients	DNA damage significantly increased in tumour patient group compared to controls	(8)
Prostate	Epithelial and stromal cells isolated from prostatic adenocarcinoma biopsies	Increase in apoptosis following treatment with gonadotropin-releasing hormone analogs	(9) and (10)
Cervical	Fibroblasts derived from vaginal biopsies of patients prior to radiotherapy	Neutral comet assay showed range of radiosensitivity, correlating with SF2 clonogenic assay	(11)
Head and neck	Epithelial cells from squamous cell carcinoma biopsies from larynx and oropharynx	Environmental mutagens caused increased DNA damage and genotoxicity in cancer group compared to controls	(12)
Head and Neck	Fine-needle aspirates taken 1, 2 or 3 min after local field radiotherapy to the index (largest) lymph node	Inter-tumour variation in radiosensitivity detected due to tumour hypoxia.	(13)
Head and Neck	Fine-needle aspirates of lymph nodes containing metastatic squamous cell carcinoma	DNA damage and repair successfully measured following irradiation	(14)
Various metastatic tumours	Fine-needle aspirates from accessible metastatic tumours	Hypoxic tumour cells successfully detected following radiation induced damage	(15) and (16)
Various metastatic tumours	Fine-needle aspirates from accessible metastatic tumours immediately following radiotherapy	Hypoxic tumour cells successfully detected following radiation-induced damage	(17)

[a]All studies employed the alkaline comet assay unless otherwise indicated.

A number of different approaches have attempted to predict the likely response of individual tumours to radiation. Currently, much work is focused on the use of microarray technology as a way to generate genetic signature profiles which will indicate the probable response of a tumour to radiation [reviewed in (21)]; this approach represents promising and revolutionary technology. However, the implementation of this technology in predictive radiation biology has yet to be validated and there are still some important limitations with regards to interpretation of gene expression data, complications raised by cell heterogeneity and expense. Moreover, assays that measure phenotypic characteristics of cells (such as DNA repair) are still necessary to validate and substantiate the findings of such technology.

Previously, a more widely used assay for predictive tumour testing was the clonogenic cell survival assay (22). In particular, the SF2 clonogenic assay, which measures the surviving fraction of tumour cells after 2 Gy X-ray irradiation, was regarded as an accurate method for measuring and predicting tumour cell radiosensitivity at clinically relevant doses (23,24). However, the main disadvantages of this assay are that a number of weeks are required to obtain a result, and many excised tumours fail to grow in soft agar (25). These limitations have stimulated an interest in developing methods that might provide a quicker, and more robust, measure of tumour radiosensitivity so that results could be considered in treatment planning.

Such methods include measuring the potential doubling time (Tpot) of the tumour (26), tumour hypoxia measured by pO_2 (27), the percentage of apoptotic or viable cells (28) and the index of thymidine and BudR labelling (29). In addition in a range of tumour types, the immunohistochemical detection of many specific proteins, e.g. p53, p21 and EGFR, have been evaluated as possible indicators of tumour cell radiosensitivity (30–32). Functional cell imaging by means of positron emission tomography or single-photon emission computerized tomography has also been employed to measure tumour cell proliferation, metabolism, hypoxia and apoptosis *in situ* in response to radiation (33,34). All these techniques show promise, but for a variety of reasons are not yet routinely implemented in clinical practice.

The comet assay is another possible predictive test and offers some distinct advantages in comparison to the techniques outlined above. It is simple and quick to do, relatively inexpensive and it does not require clonogenic cell growth as it is carried out on single-cell suspensions, all criteria which would make it appropriate for routine testing in a clinical context. Initially the neutral comet assay was used to assess radiosensitivity in a variety of tumour cell lines (35–37). However, the radiation doses required to produce measurable levels of double strand breaks (typically 20–150 Gy) were far greater than those used clinically, which also made comparison with the SF2 clonogenic assay difficult. The alkaline comet assay, however, allows radiation effects to be measured at low, clinically relevant, doses (0–6 Gy). The alkaline comet assay has been successfully used to demonstrate that radiosensitivity can be measured in a range of tumour cell lines including cervical (38,39), colon (40,41), bladder (25,42–44), prostate (40,45), renal (46), breast (47), lung (48) and ovarian (49). Since each cell line in effect represents a different individual, these studies collectively demonstrate that the comet assay is capable of detecting intrinsic differences in radiosensitivity between different tumour cell lines.

However, to be accepted as a routine test suitable for clinical use, the alkaline comet assay needs to be validated against other methods of measuring radiosensitivity and also must prove applicable to tumour cell samples extracted from cancer patients. To address the first of these issues, several studies

have compared radiosensitivity measured by the alkaline comet assay and SF2 clonogenic survival in various cell lines. Three independent studies published concurrently in 2003 demonstrated a strong correlation (all R^2 values > 0.95) between the two methods in measuring the radiosensitivity of several bladder (25,42) and colorectal (40) tumour cell lines after irradiation with <10 Gy *in vitro*, indicating that both initial DNA damage and residual damage can be used to predict for radiosensitivity in these cell lines. Similar studies have also shown a correlation between radiosensitivity measured by the comet assay and SF2 clonogenic survival following *in vitro* radiation of human melanoma (50), cervical (39) and ovarian (49) tumour cell lines. In normal fibroblasts, initial DNA damage, but not DNA repair, has also been shown to correlate with clonogenic survival (51). Although more concerted investigation in this area would allow more definitive conclusions to be made, the results of these studies would suggest that the alkaline comet assay may indeed prove to be a reliable and comparable alternative to the time-consuming clonogenic survival assay.

When cells have been extracted from excised human tumours, the alkaline comet assay has successfully identified variations in radiosensitivity in studies of bladder (42), head and neck (14), breast (6) and a range of metastatic tumours (52). These studies further demonstrate how the comet assay could support clinical decision making. Just a few cells extracted from a biopsy can be quickly assessed for their response to radiation, thereby giving additional diagnostic information to the more standard cytological techniques. A further, related approach involves investigating the extent of DNA damage and repair ability in response to radiation is measured in surrogate cells, such as lymphocytes, extracted from cancer patients (see below).

Prediction of tumour chemosensitivity

A similar problem in predicting tumour response occurs when the cancer therapy involves a chemotherapeutic drug. Drug resistance is a major obstacle to successful chemotherapy and it is not yet possible to predict the intrinsic resistance of tumour cells to a given drug or to forecast the ability of the tumour cells to develop resistance over a period of time (53). Here too, the comet assay offers a versatile candidate for a clinical assay since modifications to the standard comet assay allow the measurement of DNA cross-linking and alkylation rather than DNA strand breakage (54,55). Naturally, there are some limitations to this approach in that not all drugs produce the sort of damage that can be measured by the comet assay, but it has still proven a useful tool in assessing the sensitivity of cancer cells to particular compounds.

As with radiation, the sensitivity of cells to chemical mutagens, as well as their ability to subsequently repair the damage, can be measured using the comet assay. Several drugs, which are routinely used in cancer chemotherapy, have been investigated in a range of human cancer cell lines *in vitro*, including cisplatin (56,57), mitomycin C (43,58), tamoxifen (59,60) and epirubicin (61). The success of this approach means that investigators can explore if tumour cells can be further sensitized to the action of chemotherapeutic drugs by the introduction of other factors. For example, cells can be treated *in vitro* with combinations of drugs (61,62), with compounds that interfere with the DNA repair pathways (63) or with a combination of drugs and photo damaging agents (64,65). Thus, comet assay analysis can assess if increased

DNA damage and/or tumour cell death occurs. These *in vitro* studies on human cancer cell lines demonstrate the value of the comet assay as a tool for quickly establishing conditions in which drugs work most effectively both as single agents and in various combinations.

With this in mind, it would seem natural to apply similar techniques to tumour cells isolated from biopsy material obtained from individual cancer patients. However, few studies have taken this approach. Instead, the more common approach involves indirect measurement of the effect of anti-neoplastic drugs by assessing DNA damage in surrogate cells, such as peripheral blood lymphocytes (PBL), from cancer patients undergoing chemotherapy. This allows for easier sample collection, but is not without its problems as discussed below.

Measuring DNA damage in PBL of cancer patients

The comet assay is now a widely used tool in the field of genetic toxicology testing on human, animal and plant cells *in vitro* and *in vivo* (66). In human studies of this type, the majority of investigations examine PBL for increased levels of DNA damage, although urothelial cells extracted from urine samples and buccal cells can also serve as suitable surrogate cells. Collection of PBL is usually less invasive than target tissue and they are the surrogate cells of choice in studies where target tissue is not readily attainable (66). Hence, the comet assay has been used in assessing the exposure (often occupational) of a population to various mutagenic compounds (67). With specific regard to cancer, this approach has been used experimentally to measure the levels of basal DNA damage in PBL of cancer patients, as well as assessing if the cells are more susceptible to the DNA-damaging effects of radiotherapy or chemotherapy.

Several studies have shown that basal DNA damage is indeed increased in PBL of patients suffering from a variety of different cancers, including head and neck (68), breast (69–71), renal (72), oesophageal (2), bladder (73), ovarian (3) and lung (74). Many of these studies and others have also extracted PBL from cancer patients (usually prior to radiotherapy or chemotherapy) and exposed them to DNA-damaging agents *in vitro* to assess if susceptibility to DNA damage and subsequent repair capacity is significantly different from control samples (Table II).

With respect to radiation, several studies have demonstrated higher initial and/or residual DNA damage and lower repair rate following irradiation of PBL *in vitro* as compared to controls (68,69,74–76). Likewise, several studies have shown similar increases in DNA damage and decreases in repair efficiency in response to *in vitro* treatment with chemical mutagens such as doxorubicin (77), *N*-methyl *N*-nitro *N*-nitrosoguanidine (70), bleomycin (78,79) and benzo(*a*)pyrene diolepoxide (72).

Furthermore, many other studies have examined PBL of cancer patients who are undergoing chemotherapy or radiotherapy to see if the effect of radiation or anti-neoplastic drugs results in lymphocyte DNA damage *in vivo*; this approach is used as a surrogate indicator of how the tumour cells may be affected (Table III). These studies have shown that DNA damage is increased and/or DNA repair capacity is decreased in PBL samples from patients with a variety of cancers.

In evaluating these studies, the critical issue is the judicious interpretation of the data generated by the comet assay. It must not be forgotten that PBL are surrogate cells and therefore

Table II. Comet assay studies investigating effects of *in vitro* treatment of PBL obtained from cancer patients[a]

Tumour type	Details of study	Damaging agents	Result	Reference
Squamous cell carcinoma of head and neck ($n = 82$)	Patients: 34 M, 4 F, age 44–78; controls: 17 M, 27 F, age 13–78	*In vitro* exposure to radiation; 2 Gy	Cancer group contained significantly more patients, whose irradiated PBL showed high DNA damage, low repair rate and high non-repaired DNA damage level	(68)
Breast (primary ductal cell carcinoma) ($n = 6$)	Patients: 3F, age 47–72; controls: 1 M, 2 F, age 40–45	*In vitro* exposure to radiation; 0–4 Gy	Slower repair capacity and higher residual DNA damage in cancer group compared to controls	(75)
Breast ($n = 140$)	Patients: 70 F, mean age 52.44 ± 11.31; controls: 70 F, mean age 53.81 ± 9.61	*In vitro* exposure to radiation; 6 Gy	Significantly higher DNA damage at $T = 0$ and $T = 10$ min after radiation in cancer group compared to controls	(69)
Breast and Hodgkin ($n = 72$)	Breast cancer: 28 F, age 32–77; Hodgkin's disease: 11 M, 9 F, age 32–63; controls: 12 M, 12 F, age 21–62	*In vitro* exposure to radiation; 2 and 5 Gy	Increased levels of residual DNA damage at 30 and 60 min after radiation in breast cancer patients, but not in Hogdkin's patients. Defective DNA repair correlates with patients showing previous over-reaction to radiotherapy	(76)
Breast (primary invasive ductal cell carcinoma) ($n = 102$)	Patients: 50 F, age 48–76; age-matched controls: 52 F.	*In vitro* exposure to doxorubicin; 0.5 µM	Higher levels of damaged DNA and decreased DNA repair efficacy in PBL of cancer group compared to controls	(77)
Breast ($n = 397$)	Patients: 88 F, age 18–70; first-degree relatives: 188 F, age 18–70; controls: 121 F, age 18–70	*In vitro* exposure to *N*-methyl *N*-nitro *N*-nitrosoguanidine; 0.025 µM	A significant increase in DNA damage observed from control group to relative group and from relative group to cancer group	(70)
Lung ($n = 66$)	Patients: 21 M, 15 F, age 47–73; controls: 14 M, 16 F, age 40–78	*In vitro* exposure to radiation; 3 Gy	Higher levels of DNA damage in irradiated PBL of cancer group compared to controls	(74)
Lung (non-small cell) ($n = 340$)	Patients: 117 M, 43 F, mean age 60.5 ± 9.2; controls: 103 M, 77 F, mean age 52.2 ± 13.0	*In vitro* exposure to bleomycin; 20 µg/ml	Significantly higher DNA damage in cancer group than controls following bleomycin treatment	(78)
Renal cell carcinoma (RCC) ($n = 386$)	Patients: 116 M, 77 F, mean age 59.12 ± 10.46; controls: 116 M, 77 F, mean age 60.81 ± 9.86	Separate *in vitro* exposure to radiation, 1.5 Gy and benzo(*a*)pyrene diolepoxide; 2 µM	Significantly higher DNA damage in cancer group than controls following both treatments; associated with significantly increased risks of RCC	(72)
Various cancers ($n = 66$)	Patients: 14 M, 19 F, age 35–79; controls: 14 M, 19 F, age 37–85	Separate *in vitro* exposure to UVC radiation; 1.5 J/m and bleomycin, 20 µg/ml	DNA repair capacity was significantly lower in cancer group than controls following both treatments	(79)

[a]*n*, number; M, male and F, female. All studies used the alkaline comet assay.

damage detected in these cells may not necessarily reflect the damage in tumour cells or any other target tissue, although it is worth noting that some studies, using methods other than the comet assay, have shown good correlation between patient response to chemotherapy and DNA adducts (89,90) and DNA repair capacity (91) in blood cells. Also, as most of the studies above acknowledge, basal and residual levels of DNA damage in PBL can be influenced by many external factors, such as smoking, diet, medication, air pollution and exercise among others, resulting in a wide intra-individual and inter-individual variability (67). Such a range of confounding factors has the effect of undermining the worth and appeal of the comet assay as a predictive clinical test. Thus, it seems clear that until a standard protocol is agreed upon and multi-laboratory validation trials are carried out for quality control, it will be difficult for the comet assay to gain widespread clinical acceptance as a reliable method for measuring DNA damage in PBL caused by exposure to radiotherapy or anti-neoplastic drugs (67,92).

Measurement of tumour hypoxia

Tumour hypoxia has been linked to poor tumour response to both radiotherapy and chemotherapy and is also associated with a poor outcome following surgery (93). The ability to measure hypoxia in tumours can provide important predictive information about probable tumour response, thereby allowing the individualizing of treatment according to hypoxic status (94). Currently, one of the most common methods for measuring tumour hypoxia is by using an Eppendorf oxygen microelectrode to measure pO_2 levels in solid tumours (95). However, this approach has difficulty in distinguishing between hypoxic and necrotic regions and is limited to accessible tumours (93). The comet assay can be used to measure tumour hypoxia on any excised tumour biopsy, based on the premise that radiation produces approximately three times more DNA damage in well-oxygenated as compared to hypoxic cells (6,15). Cells can be obtained by fine-needle

Table III. Comet assay studies investigating the effect of chemotherapy and radiotherapy on PBL of cancer patients *in vivo*[a]

Tumour type	Details	Treatment(s)	Result	Ref
Breast (invasive ductal cell carcinoma) ($n = 102$)	Patients: 50 F, age 48–70; healthy, untreated controls: 52 F	Cyclophosphamide, methotrexate and 5-FU (i.v.)	Increased DNA damage and decreased repair efficiency in cancer group compared to controls	(77)
Various ($n = 47$)	Patients: 8 M, 16 F, age 35–79; healthy, untreated controls: 7 M, 16 F, age 27–85	Fractionated radiotherapy (up to 50 Gy)	Increased DNA damage in cancer group compared to controls. Inter-individual variation to radiotherapy also observed	(80)
Breast (invasive ductal cell carcinoma) ($n = 8$)	Patients: 8 F, age 25–50	Doxorubicin and ifosfamide (i.v.)	DNA damage detected in PBL at 24 and 48 h—related to levels of the ifosfamide metabolite 4-hydroxyifosfamide	(5)
Various ($n = 10$)	Patients: 2 M, 8 F, age 37–58	Combinations of i.v.: adriamycin, leucovorin, vincristine, cisplatin, prednisone, cyclophosphamide, methotrexate and 5-FU	Significant DNA damage in PBL after treatment with various anti-neoplastic drugs, compared to pre-treatment measurements	(81)
Metastatic colorectal cancer ($n = 5$)	Patients: 2 M, 3 F, age 57–70	Oxaliplatin (i.v.)	Successful detection of cross-link damage and subsequent repair. Inter-individual variation shown in both initial DNA cross-link damage and repair	(82)
Metastatic melanoma ($n = 12$)	Patients: 7 M, 5 F age 39–82	Temozolomide (oral)	Increased, but not significantly, DNA damage following administration of drug, compared to pre-treatment measurements	(83)
Breast ($n = 32$)	Patients: 32 F, no age range given	Radiotherapy to whole breast (25×2 Gy), followed by irradiation of tumour bed (5×2 Gy)	Comet assay distinguished between patients with acute and average radiosensitivity as determined by SF2 assay. Slower DNA repair observed in radiosensitive patients	(84)
Various ($n = 25$)	Patients: 16 M, 9 F, age 1–59; healthy, untreated controls: 5 M, 2 F, age 22–50	Various anti-neoplastic drugs including doxorubicin and cisplatin (method of administration not stated)	Increased DNA damage in cancer group compared to controls. Decreased repair of cisplatin adducts in cancer group compared to controls	(85)
Nasopharyngeal ($n = 9$)	Patients: 9, age 26–54	Fractionated radiotherapy (up to 68 Gy)	Dose-dependant increase in DNA damage, significant at higher radiation doses	(86)
Malignant melanoma ($n = 39$)	Patients: 22 M, 17 F, age 26–81	Tamoxifen and dacarbazine (i.v.)	Successful detection of DNA damage and repair following dacarbazine treatment. Wide inter-individual variation in PBL DNA strand breakage	(87)
Ewing's sarcoma ($n = 13$)	Patients: 10, sex and age not provided; healthy controls: 3	Ifosfamide (i.v.)	Successful detection of DNA cross-link damage in patient group	(88)

[a]*n*, number; M, male; F, female and i.v., intravenous. All studies employed alkaline comet assay.

aspirate biopsy following *in vivo* irradiation of the tumour and the assay is not affected by necrosis. This approach has been used to successfully measure tumour hypoxia in radiotherapy patients with head and neck tumours (13), breast (6) and a range of metastatic tumours (17,52). However, there are some limitations in that radiation doses in excess of 3.5 Gy are required immediately before the biopsy in order to detect hypoxic cells with the comet assay and there is a risk of contamination from circulating white blood cells (52).

While both the oxygen microelectrode and comet assay techniques are capable of identifying hypoxic tumours, studies comparing the two techniques in human tumours have failed to show a consistent correlation (13,16,17,52), which presumably reflects the intrinsic differences in how they measure hypoxia. Although there is a recognition that hypoxia is a significant factor affecting both tumour response to treatment and patient

survival, there is currently no consensus as to the best way to measure hypoxia (94). The comet assay offers one method to address this issue but the results are unlikely to be accepted unless corroborated with other methods of hypoxic cell determination.

Clinical applicability of the comet assay

A recent review by Taube *et al.* (96) states that a new diagnostic tool must pass three major tests before it is adopted for routine clinical use: (i) it must be robust and reproducible, (ii) the clinical value to the patient must be proven and (iii) the clinical community must be convinced of the value and benefit of such a tool. These pertinent points should act as a guideline in focusing our collective efforts in seeking clinical acceptance for the comet assay as a diagnostic technique and predictive

test for response to radiotherapy/chemotherapy in the management of cancer and for its application to the management of other disorders. Indeed, these points also apply to other potential methods for predictive assays and a series of stringent guidelines has already been produced for tumour marker prognostic studies to help minimize poor study design, non-standardized assays that lack reproducibility and inappropriate or misleading statistical analyses (97). A similar list of guidelines for comet assay studies would help to encourage more transparent and thorough investigations, thereby allowing relevant and unambiguous information from each study to be available to others in a way that will inevitably improve the quality, significance and applicability of comet assay data and results.

In the meantime, it may prove fruitful to make a concerted effort to qualify and collate all existing published comet data into a central database allowing pooled analysis of previous work. Such an approach has successfully resulted in establishing that high chromosome aberration frequency in PBL was a reliable predictive biomarker for cancer incidence, a conclusion that was based on the work of a coordinated, international, multi-centre cohort study which analysed a joint database comprised of data on 3184 subjects from previous studies (98). A corresponding approach for comet assay studies might also yield similarly pertinent information. Indeed, it is worth noting that an inter-laboratory consortium has already been established in an attempt to implement standard, reliable protocols (including the comet assay) for sample preparation and analysis of low levels of 8-oxo-7,8-dihydro-2′deoxyguanosine in lymphocyte DNA (99). With such collaboration and teamwork already in place, an excellent opportunity exists to establish a separate inter-laboratory committee focused on the comet assay and its potential in the clinical management of cancer. In addition, efforts must be made wherever possible to forge and strengthen collaborations with colleagues in hospitals and clinical laboratories, not just with the aim of obtaining useful cell samples, but with a view to establishing the worth of the comet assay in the eyes of the clinical community. Without support and backing from oncologists, clinicians and regulatory authorities, the comet assay will struggle to gain acceptance as a routine diagnostic tool in cancer management.

Conclusions

The studies discussed in this paper demonstrate that the comet assay can successfully measure DNA damage and subsequent repair in a variety of human tumour cells following exposure to both radiation and mutagenic compounds. It is a sensitive, rapid and versatile technique, lending itself well to the study of a range of DNA-damaging agents, such as those used in cancer therapy. However, despite this, the comet assay will not, and indeed should not, be used in the management of cancer or any other disease unless fundamental issues relating to experimental validation, standardization and data interpretation are addressed.

Acknowledgements

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