Atlas of Genetics and Cytogenetics in Oncology and Haematology

OPEN ACCESS JOURNAL **INIST-CNRS**

Deep Insight Section

Cytogenetic biodosimetry for accidental emergency irradiation exposure preparedness, in particular merit of the use of drug-induced premature chromosome condensation (PCC) with calyculin A

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Published in Atlas Database: July 2012

Online updated version : http://AtlasGeneticsOncology.org/Deep/BiodosimetryID20114.html
DOI: 10.4267/2042/48476

Abstract

More than one year has past since the hazardous nuclear fuel core melting out accident at Fukushima No.1 Nuclear Energy Plant by attacking giant earthquake and following tsunami on 11th March 2011. This nuclear plant accident is so obviously the most serious radiation exposure hazard since the Chernobyl nuclear plant explosion in USSR (now in Ukraine) 1986, that recognizes the people over the world of the plausible scenario of threaten of nuclear hazard accident caused by natural disaster, nuclear terrorism attack or assault of nuclear weapon. In the situation of emergency irradiation exposure accident happened, the actions which must be first undertaken are (1) determine the event level and estimate the precise irradiation dose as quick as possible, (2) prevent the people both citizens and workers from external and internal irradiation exposure and contamination and (3) if casualties exist, triage diagnosis should be done to perform required medical management appropriate for the patients severely or lethally radiation exposed as rapid as possible. For the purposes, precise dose estimation system establishment should be requested and prepared. However, in particular in case of the emergency irradiation accident, body-worn dosimetry equipment such as film badge is useless for assess the exposure dose of public citizens because people usually don't put on these tools on body. Biological dosimetry (biodosimetry) is a suitable way for this purposes and cytological biodosimetry methodology is most popular and has been used for long time. It is now therefore seems the adequate time to consider the importance of preparedness of biodosimetry based dose assessment system for the radiation exposure accident caused by natural disaster, terrorism or war, so here we present short review of the cytogenetic biodosimety method by means of scoring the radiation induced chromosome aberrations, in particular for the high dose emergency radiation exposure accident.

Introduction

The threat of nuclear crisis suddenly overwhelmed Japan last year. A giant earthquake (magnitude 9.0) and big tsunami attacked eastern area of Japan 11th March 2011 (Higashi-Nihon Dai-Shin-sai), and more than 20 thousands of people died and 2 thousands of people are still missing. The disaster simultaneously attacked Fukushima Daiichi Nuclear Power Plant (Fukushima No.1 Nuclear Power Plant) of TEPCO (Tokyo Electric Power Company). Four of six nuclear reactors have been seriously damaged; some of them resulted in core melted. Enormous amount of nuclear fission radioactive products have been scattered over wide area, resulted in radioactive contamination of atmosphere, soil and water. Fortunately, no victims eventually harmed or died caused by irradiation exposure, a lot of habitants around the nuclear plant have been forced to evacuate from there and they are still not able to come back to home. The hazard level was finally pulled up to the worst "Level 7" on INES scale (International Nuclear Event Scale), which is the same as that of the Chernobyl nuclear plant explosion

in USSR April 1986. The Fukushima nuclear plant accident has arisen a great public concern not only in Japan but also in over the world that the threat of great scale irradiation exposure accident and radioactive contamination will happen anytime and anywhere in the world. The accident also reminds the importance of protection and medical management system against irradiation exposure.

Dose estimation in case of emergency irradiation exposure accident

In case of emergency irradiation exposure accident, most important actions must be undertaken urgently are (1) protection of people both public citizens and radiation workers from exposure of external and internal irradiation, (2) triage in mass to classify the lethal dose casualties, the subjects exposed discrete amount of dose and low/no dose exposed persons and (3) provide necessary medical management for life save the severe injured casualties as quick as possible (Bland, 2004; Blakely et al., 2005; Coleman et al., 2009; Göransson Nyberg et al., 2011). To accomplish, an accurate and immediate irradiation dose assessment for human body must be done for decision and choice of appropriate medical management, in particular in case of the victims are supposed to be received high dose irradiation exposure which may cause severe or lethal damages to human bodies (from 2-3 Gy to several Gy of γ-irradiation and sometimes over 10 Gy). In case for the exposure accident on occupational workers in irradiation control area, individual exposure dose is monitored either by area monitoring system or by body-worn monitoring tools such as pocket dosimeter, film badge dosimeter or thermo-luminescence dosimeter (TLD).

These equipments utilize the radio-physical or radio-chemical methodologies to measure irradiation doses. The measured dose by means of these tools is in principle only for external exposure irradiation and therefore not for internal exposure for body, which can give more serious damages in cells or organs than external irradiation. The measured dose by these tools does not tell the actual damages introduced in body.

More problematic situation is that accidentally exposed public citizens do not usually wear these monitoring tools in emergency irradiation exposure accidents. Therefore, the dose estimation for public citizens is practically impossible by means of body-worn dosimetry tools. Therefore, irradiation dose estimation in case of emergency radiation accident must be exploited by alternative methodologies, i.e. biodosimetry.

Biodosimetry for irradiation exposure event

Biodosimetry or biological dosimetry is a dose assessment method by means of observe the symptom/phenomenon appear after irradiation or measure physical/chemical/biological changes or damages introduced in cells, organs or body given by ionizing irradiation. The number of empirical knowledge or methodologies have been known and established. It is well known that various clinical symptoms tightly associated with ionizing irradiation exposure is called ARS (Acute Radiation Sickness/Sign/Syndrome) (House et al., 1992; Anno et al., 2003; Waselenko et al., 2004; Blakely et al., 2005) (for example skin rash or reddish, skin barn, leukopenia, purpura, alopecia, diarrhea or nausea/vomiting, dizziness, seizures, loss of consciousness and others appears after irradiation exposure as dose dependent manner). Dose dependency of these symptoms is tightly associated with the radiation sensitivity of the responsible organs/tissues, i.e. bone marrow system, hair roots, gastrointestinal tract system or central nervous system. Table 1 shows the major ARS and approximate dose dependency. Based on ARS, given dose can be assessed roughly by onset of characteristic symptoms associated with the dose. However, the dose response ranges of each ARS is somehow broad as shown in Table 1 and the range also varies between individual and it is, therefore, not useful to estimate the received doses precisely. Time lapse until onset of symptoms appear after irradiation exposure is also depends on dose as shown in Table 2; usually it takes more longer time along with the dose decreasing until symptoms appear, thus it is also drawbacks for quick dose estimation excepting for extreme high dose (more than 10 Gy) irradiation case (Swartz et al., 2007). For example as to skin rash sign display, it varies from a couple of days to couple of weeks after irradiation exposure along with the dose decreasing. Nausea and vomiting are one of typical sign of ARS, but they will be associated and enhanced with other factors such as trauma or panic syndrome during accident.

Therefore, determination of given dose based on ARS is usually less informative, rapidness or reliability.

Dose received* (γray equivalent)	Affected organ and symptoms	Survival time
5-10	Bone marrow system (Lymphopenia, Purpura, Breeding)	<3 weeks
10-70	Gastrointestinal tract system (Diarrhea)	< 1 week
70-200	Central Nervous system (Nausea, Vomiting, Seizure, Loss of Consciousness)	< 3 days
>200	Molecular death	< several hours

Table 1. Dose dependency of acute radiation syndromes (ARSs) associated with the affected organ and average survival time after exposure by irradiation.

ARSs / given Dose		Vomiting	Diarrhea	Headache	Consciousness
Mild	1-2 Gy	> 2 hr	-	Slight	-
Moderate	2-4 Gy	1-2 hr	-	Mild	-
Severe	4-6 Gy	< 1 hr	> 24 hr	> 24 hr	-
	6-8 Gy	< 30 min	1-3 hr	3-4 hr	Possible
Lethal	> 8 Gy	< 10 min	< 1 hr		Sec - Minutes

Table 2. Dose dependency of ARSs and time of onset (IAEA, 1998; Göransson Nyberg et al., 2011).

Blood cell count is also a good indicator for the exposed dose. Circulating leukocytes or lymphocyte (WBCs; white blood cells) are very sensitive to ionizing irradiation, thus they quickly disappeared from the circulating peripheral blood following exposure of irradiation (Dainiak et al., 2003). Although the disappearance of WBCs from peripheral circulation is indeed dependent on received dose, individuals factors such as age, sex, health condition, smoking deeply influence, and therefore it is difficult to estimate irradiated dose precisely, as well. Therefore, hematological scoring also will not be suit for rapid and precise dosimetry method. Haematopoietic cell circulation kinetic is also complex. Lymphocyte or leukocyte counts decreased after irradiation, then they usually recover, but will not increase or never return after high dose exposure. This is also make it difficult for calculate the given dose by kinetic of haematopoietic cell data.

Detection and measure of radioactive nuclide such as ^{24}Na in body fluids artificially produced by neutron irradiation exposure is also available (Ishigure et al., 2001), which is based on the following neutron capture reaction:

$$^{23}Na + n \rightarrow {}^{24}Na$$

^{23}Na is natural occurring stable isotope, which is rich in living organism, exists as Na^+ ion. ^{24}Na is absolutely not exit naturally in human body fluid and therefore the detection of ^{24}Na in body fluids is a specific evidence of neutron exposure. However this method is only available for neutron irradiation case. Half-life of ^{24}Na is about 15 hours, so it is not applicable for dose assessment for long time after exposure events.

The electron paramagnetic (EPR) tooth (Swartz, 1965; Swartz et al., 1965) is used for estimate the exposure dose by means of measure the amount of CO_2 radicals in the solid matrix components such as tooth enamel, fingernail or extracted bone after irradiation events.

As the CO_2 radical is very stable, it allows the given dose be estimated for long time after irradiation and dose response sensitivity is linearly for wide ranges (from 100 mGy to 300 Gy). However invasive manipulation for sampling specimens (i.e. the extract of tooth, excepting the infant for replacing milk tooth) is disadvantage. Recently in vivo method using tooth or fingernail has been developed to overcome this problem (Swartz et al., 2007; He et al., 2011).

Other dose assay protocols using newer molecular biology biomarker method have been recently developed such as γH2AX detection after DNA damage (Rogakou et al., 1998; Rogakou et al., 1999; Paull et al., 2000), microarray analysis of gene expression pattern following irradiation (Amundson et al., 2001), or proteomic or metabolomic profiling analysis (Nicholson et al., 2002; Oberemm et al., 2005).

The details of these methods is beyond the scope of this article, but combined use of these methodologies should be much more useful in biodosimetry, in particular the case of emergency accident in which rapid and precise dose estimation for triage decision should be required.

Cytogenetic biodosimetry for irradiation exposure event

Biological dosimetry by means of assaying chromosome aberration induced by ionizing irradiation (cytogenetic biodosimetry) is an useful and established dose estimation method as recognized fills gap in dose assessment in case such as where the irradiation exposed persons not wearing dosimeter equipment (Lloyd, 1984; Beninson et al., 1986; Catena et al., 1993; IAEA, 2001; IAEA, 2011). It has been well known since 80 years ago that ionizing irradiation induces mutation in living organism (Muller, 1927) and chromosome aberrations are introduced by ionizing irradiation (Sax, 1941), therefore it has a long history to score chromosome aberration for assess the irradiation dose since mid-1960s. Dicentric assay use of mitotic chromosomes obtained from mitogen activated peripheral blood lymphocytes has been only cytogenetic dosimetry method for many years, and dicentric assay therefore has been as the "golden standard" of cytogenetic biodosimetry (Hoffmann and Schmitz-Feuerhake, 1999; IAEA, 2001). Cytogenetical biodosimetry using chromosomes obtained from peripheral blood lymphocyte has following several merits - something ideal system; (1) Easy of sampling small 10 to 20 ml volume of peripheral venous blood as mostly non-invasive way and culturing blood lymphocytes using T-cell lymphocyte mitogen PHA (Nowell, 1960; Carstairs, 1962) and (2) following chromosome preparation (Moorhead et al., 1960; Nowell and Hungerford, 1960) is well established and very simple technique, (3) Result comes within relatively short time (48 hours at first mitosis after start of lymphocytes culture), (4) Do not require special or expensive instruments, (5) Aberration of chromosomes tell the actual cellular damages given both from external or internal irradiation exposure, (6) Chromosomes record total accumulated damages ever since the exposure accident. It means that dose estimation for chronic or repeated exposure events is applicable. (7) in vivo arrested peripheral lymphocytes lives over several years in the body (Buckton et al., 1967; Bogen, 1993; McLean and Michie, 1995), some population of lymphocytes live over 10 years in vivo (Awa, 1991), therefore it promises to estimate doses for long time since the exposure event (Ramalho et al., 1995), (8) Induction of chromosome aberration is highly sensitive to ionizing irradiation and well dependent on exposed dose over wide range. The possible minimum estimation dose is known about 2-10 mGy for neutron exposure (Vulpis et al., 1978; IAEA, 2011) or 50-100 mGy for γ-ray exposure (IAEA, 2001; IAEA, 2011), which indicates that cytogenetic biodosimetry has a sufficient dose sensitivity for coverage both acute and chronic irradiation exposure. However, cytogenetic biodosimetry by means of chromosome preparation by the conventional mitotic blocking method use of colcemid has several drawbacks.

The dicentric assay is reliable only up to 5 Gy due to saturation of the dose response (Lloyd and Edwards, 1983; Göransson Nyberg et al., 2011). Further practical problem is that the irradiation dose increase to higher in particular than 10 Gy, the damaged cells arrest cell cycling in G1 or G2 phase and do not enter in mitosis or lost before mitosis during apoptic cell death. So it is difficult or impossible to obtain mitotic chromosomes from the cells exposed with high dose of irradiation. Therefore, it has long time been limitation of cytogenetic biodosimetry over several Gy of exposure cases. In addition, more than 20 years ago, over several Gy exposure dose of irradiation on whole body was lethal to human bodies and it was impossible to save victims, thus it was not practically required for the cytogenetic biodosimetry to assure estimating radiation dose over several Gy. However, with the recent development of medical technology such as stem cell transplantation and available of cytokines such as G-CSF, it has become possible to save the victims who irradiated whole body over several Gy (Lloyd, 1997). Therefore, the request has been raised for developing the cytogenetic biodosimetry method that promises the estimable dose for over 10 Gy (~LD50/60 for humans and upper limit of conventional cytogenetic biodosimetry using colcemid block method). Premature chromosome condensation (PCC) technique is a possible overcome alternative way to this limitation for preparing chromosomes from heavily damaged cells by high dose irradiation, because PCC allows the interphase chromosomes to be condensed and observed like as mitotic condensed chromosomes as well.

Premature Chromosome Condensation (PCC): cell fusion-mediated PCC

Premature chromosome condensation (PCC) technique (Johnson and Rao, 1970) has been widely used in cytogenetic fields, particularly in radiation biology because it allows interphase nucleus to be condensed like as mitotic chromosomes. PCC forces to condense chromosomes even in irradiation damaged cells that arrested before mitosis (Hittelman and Rao, 1974; Waldren and Johnson, 1974; Cornforth and Bedford, 1983). PCC is conventionally induced by fuse the recipient cell with mitotic arrested inducer cell use of fusogenic agent such as virus (Johnson and Rao, 1970) or polyethylene glycol (PEG) (Pantelias and Maillie, 1983). However, the cell fusion procedure to induce PCC (cell-fusion mediated PCC) is technically demanding. In addition the PCC induction efficiency is highly depend on viability of virus or PEG quality which used in cell fusion.

Thus, it has limited the use of fusion-induced PCC in non-authorized researchers or laboratories. In 1995,

drug-induced PCC using okadaic acid or calyculin A (Gotoh et al., 1995), specific inhibitors of protein phosphatases opened the new way of use PCC method in wider area of cytogenetics including radiation biology and chromosome research. Drug-induced PCC was soon applied in cytogenetic biodosimetry for assessment of high-dose irradiation, and maximum reliable estimation dose was up to 40 Gy (Gotoh and Asakawa, 1996). Drug-induced PCC is now recognized as new tool for cytogenetic biology (Gotoh and Durante, 2006). Drug-induced PCC has now been used in wide area of cytogenetic field (Srebniak et al., 2005; Deckbar et al., 2007; Gotoh, 2007; Kramer et al., 2008; Gotoh, 2009; Gotoh, 2011; Miura and Blakely, 2011).
Drug-induced PCC protocol is very simple and described elsewhere (see for example Gotoh, 2009). Elucidate the phenomenon underlining PCC will advance to understand the mechanism of chromosome condensation (Vagnarelli, 2012).

Cytogenetic biodosimetry by means of drug-induced PCC use with calyculin A

Drug-induced PCC allows the interphase nuclei to be observed as condensed form of chromosomes like as mitotic chromosome as well as fusion-induced PCC (Gotoh and Durante, 2006), therefore, drug-induced PCC has been used widely in radiation biology field to analysis of radiation induced chromosome damages in interphase nuclei (Durante et al., 1998; Gotoh et al., 1999).
Use of recently developed drug-induced PCC (premature chromosome condensation) technique further facilitates the cytogenetic biodosimetry, as follows; (1') Drug-induced PCC is very simpler and easier methodology (Gotoh, 2009) even than conventional colcemid block protocol.
Therefore, it is very easy to learn and skill in technique.
(2') Maximum estimation dose of cytogenetic biodosimetry has been long time limited up to several Gy by conventional colcemid block protocol for obtain mitotic chromosomes.
Gotoh and Asakawa first overcame this limitation using drug-induced PCC (Gotoh et al., 1995) coupled use of in situ chromosome painting method, and showed that the possible maximum estimable dose was markedly pulled up to 40 Gy of γ-rays exposure (Gotoh and Asakawa, 1996).
Figure 1 shows the severely damaged chromosomes seen after 40 Gy of γ-irradiation. Many of very short-sized fragmented chromosome piece, very-long sized abnormal chromosome are easily seen, that is quite eccentric appearance which typically seen in high dose irradiated chromosomes (Gotoh and Tanno, 2005).

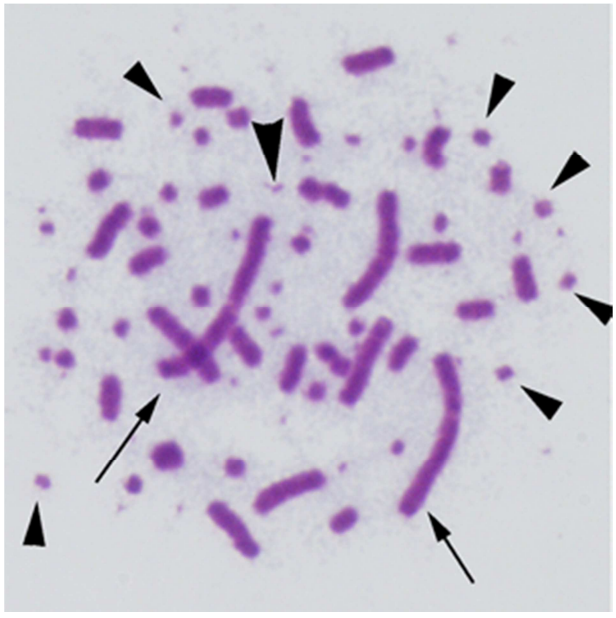

Figure 1. Forty Gy γ-irradiated human peripheral blood lymphocytes (PBLs) chromosomes obtained by means of calyculin A induced premature chromosome condensation (PCC) method. As easily recognized, chromosomes are severely damaged; presumably no intact chromosome remains. Total chromosome number increased to 78 (control 46) involving very long sized chromosomes (shown by arrow), small pieces of chromosomes (shown by arrowhead) and many of very small sized chromosome fragments (shown by large arrowhead). Methods: human peripheral blood lymphocytes (PBLs) were irradiated with 40 Gy of γ-rays. Forty eight hours after irradiation, PBLs were forced to condense using 50 nM of calyculin A, then chromosome spreads sample was prepared and analyzed as described previously (Gotoh and Asakawa, 1996; Gotoh and Tanno, 2005; Gotoh et al., 2005).

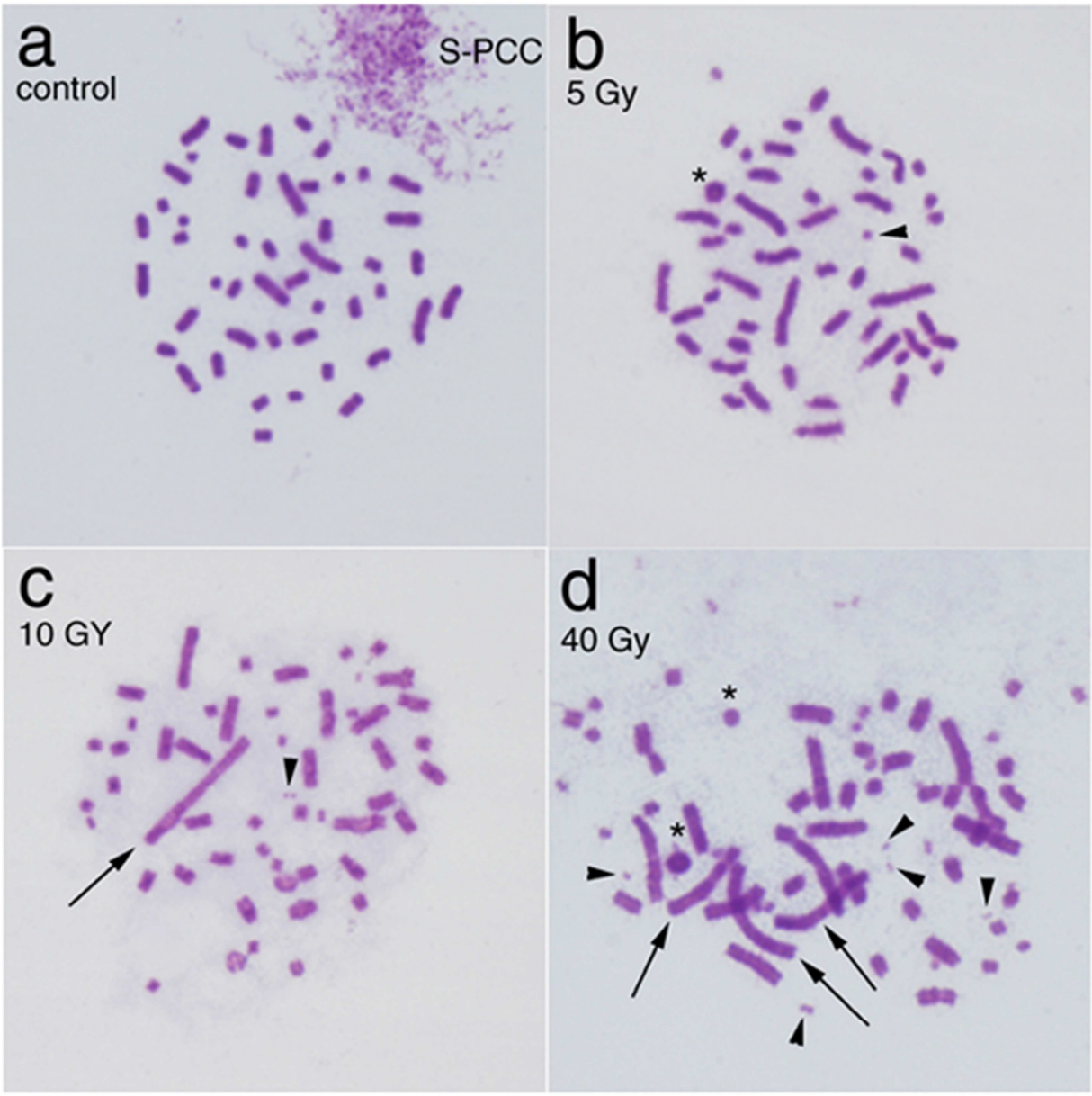

Figure 2. Typical appearance of chromosome aberration seen after exposure of different dose of γ-irradiation. (a) Control (no irradiation). Regular sized pair of intact chromosome are seen. "Pulverized" appearance of chromosomes are seen at upper right area, which is characteristic shape of S-PPC (PCC of S-phase nuclei) (Gotoh et al., 1995; Gotoh and Durante, 2006; Gotoh, 2007) but not aberrant chromosomes influenced by irradiation. (b) 5 Gy of irradiation. Chromosomes seem almost normal like as control, but a possible ring chromosome (shown by asterisk) and short fragment chromosome (shown by arrow head) are seen. (c) 10 Gy of irradiation. Chromosomes show strange appearance (irregular size, number). One chromosome is very long (shown by arrow) and very small size like as double minutes (DMs: shown by arrowhead) are seen. (d) 40 Gy of irradiation. Chromosomes are quite strange appearance even than 10 Gy irradiated chromosome, which is easily seen a glance by eye. Several chromosomes show long length (shown by arrows), in contrast many short fragments (shown by arrow heads) are seen. Two ring chromosomes are also seen (shown by asterisks). Methods are as same as described in Figure 1.

The usefulness of drug-induced PCC method for estimation of large dose exposure accident has actually been acknowledged in case of emergency exposure of JCO criticality reaction accident at Tokai-Mura Japan 1999.

Irradiation dose estimation for 3 severely exposed workers was done by means of this method and over 20 Gy of γ-ray equivalent exposure dose was estimated for most injured victim (Hayata et al., 2001). Since then, Drug-induced PCC has been actually utilized in cytogenetic biodosimetry involving high-dose or high LET irradiation exposure (Gotoh and Tanno, 2005; Gotoh et al., 2005; Lamadrid et al., 2007; Wang et al., 2007; Wang et al., 2009; Balakrishnan et al., 2010; Lamadrid et al., 2011). (3') The PCC index is usually substantially high (often over 50%), which is compared

with mitotic index by means of colcemid blocking method (usually 2-3%). The high index number makes much easier to observe and score sufficient number of chromosome spreads.

In particular, this advantage is also useful in assesses low dose exposure, because scoring of a lot number of chromosome spreads (sometimes over 10000 spreads per sample) is absolutely required for low absorbed dose estimation with substantial statistical analyzing (IAEA, 2001; IAEA, 2011). It was usually required to prepare a lot of numbers of glass slides and to observe them. Drug-induced PCC quite reduced the number of glass slide that should be scored. (4') To make precise dose estimation for triage decision and medical management for the casualties, nonetheless a substantial number of chromosomes should be scored and analyzed. Most critical point of triage is making decision whether the casualty is received over lethal dose or under the dose rescues the lives. It is possible to estimate the dose approximately and quickly seeing the PCC chromosome samples by eye. Because, as the irradiation dose increased over 10 Gy, a characteristic strange shape of chromosomes like as 'rogue' chromosomes (Neel et al., 1992; Sevan'kaev et al., 1993; Mustonen et al., 1998) are appeared, which is easily recognized by look a glance by eyes. Figure 2 shows the chromosomes appearances irradiated different doses from 0 Gy (no irradiation) up to 40 Gy of γ-rays. Under 10 Gy of γ-irradiation, chromosomes are not so strange shape (Fig. 2b 5 Gy) and similar as control intact chromosomes (Fig. 2a 0 Gy). When the cells are irradiated over 10 Gy of γ-rays, chromosomes are very different from control, involving abnormally long-sized chromosomes (shown by arrows in Fig. 2c 10 Gy), a lot of fragmented chromosomes are recognized (shown by arrowheads in Fig. 2c). The strange appearance is much more clear as irradiation dose increased (Fig. 2d 40 Gy), several long sized chromosomes (shown by arrows), number of fragmented chromosomes (shown by arrowheads) and ring chromosome (shown by asterisk) are seen. So, it is easy to estimate whether exposure dose for casualty is over 10 Gy or less. (5') Thus, cytogenetical biodosimetry coupled use of drug-induced PCC is a possible ideal tool for dose estimation for both emergency exposure accident and assessment of late or chronic irradiation effect, as the methodology covers from low dose range (10 mGy) to lethal level of high dose range (40 Gy maximum) (Gotoh and Asakawa, 1996; Gotoh and Tanno, 2005; Gotoh et al., 2005).

Minimal critical points over cytogenetical biodosimetry for emergency irradiation exposure accident

It seems out of scope for discuss about the details of dose assessment system include make of triage decision and medical management for emergency radiation exposure accident, because it is highly complex system and requires administrative support. So I just want simply to describe some critical points of chromosome preparation process for practical exploit of dose estimation using cytogenetical biodosimetry in case of emergency irradiation exposure accident. Scrupulous care should be taken in sampling blood, culture of peripheral blood lymphocytes, chromosome preparation and scoring chromosome aberration. No error must be accepted throughout process, because repeated sampling of blood is absolutely not expected in particular from heavily damaged victims. It is ideal for carry out whole course in individual local facilities, however, these procedures are somehow technical demanding and it is usually still limited to execute every step in many local hospitals. Therefore, wide area covered framework for support cytogenetical biodosimetry monitoring system should be established. Preparation of chromosome specimens is depending on skill of individual facilities, thus minimum executable practice level will be settled based on individual facility capacity. Follows are for example, (1) lowest level. Rapid elimination of lymphocytes from peripheral blood immediately begins after irradiation exposure and sometimes never recovers particularly in case of high dose exposure. Therefore, sampling of the casualty's blood must be undertaken immediately on the accident spot ideally or as rapid as possible at the local hospitals in which casualty admits, then taken blood sample should be immediately transferred to central institute or service laboratory for successive culture and chromosome preparation steps, (2) moderate level. Chromosome preparation efficiency is deeply depend on lymphocytes viability as they are very sensitive to the ambient temperature (Purrott et al., 1981; Gotoh and Tanno, 2005). Most serious temperature change can be happen during transportation of the blood sample to second laboratory which has sufficient technique on lymphocyte culture and chromosome handling. Therefore, it is much better to carry out whole step of sampling blood, culture blood lymphocyte and chromosome sample preparation at the hospitals for avoiding temperature change through every step. If it is not possible, blood samples should be packaged with thermal insulated envelopes and do not use ice or chilling materials, because cold temperature decrease lymphocyte activity (Gotoh and Tanno, 2005). If it is possible to prepare chromosome specimen (spread on glass slides), it is much ideal to complete the sample preparation because it will be more safety to send it to the central institute for chromosome aberration analysis.

(3) Highest level. For rapid dose estimation, chromosome analysis must be done as quickly as possible following preparation chromosome sample on the glass-slides. It is therefore, best to do prepare chromosome sample and chromosome analysis integrated in the facility/hospital where the casualty admits.

To achieve cytogenetic dosimetry system efficiently and thoroughly for urgent irradiation dose estimation preparedness, (1) establish a wide scale close and tight framework to contact and communicate among local hospitals and central authorized laboratory/institute, (2) make clear and simply written documentation of protocols and manuals, and circulate them over individual facilities and (3) periodical and perpetual training program must be performed.

Conclusion

Cytogenetic biodosimetry is a simple, useful and unique irradiation dose assessment method for human body not only for dose estimation for urgent irradiation exposure accident but also for evaluate chronic or repeated exposure and assess late irradiation effect. Chromosome aberration tells actual damages given in cells or organ either by external or internal irradiation exposure, which is very unique and different from other physical or chemical methodologies. Moreover, cytogenetic biodosimetry coupled use of drug-induced PCC covers wide assessment dose range up to 40 Gy of γ-irradiation, which is substantially enough to make triage and medical managing decision for casualty in irradiation exposure accident. Therefore, it is keenly recommended to establish a wide area covering framework system of cytogenetic biodosimetry for accidental irradiation exposure preparedness, particularly in countries, which own number of nuclear energy plants. Thus, drug-induced PCC combined biodosimetry method is much more preferable for dose estimation preparedness of emergency accident.

Acknowledgement

I wish to express my deepest thank to Dr. Jean-Loup Huret for give me an opportunity to write this article in Deep Insight for the Atlas of Genetics & Cytogenetics in Oncology and Haematology.

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This article should be referenced as such:

Gotoh E. Cytogenetic biodosimetry for accidental emergency irradiation exposure preparedness, in particular merit of the use of drug-induced premature chromosome condensation (PCC) with calyculin A. Atlas Genet Cytogenet Oncol Haematol. 2013; 17(1):70-79.