REPORTS OF PRACTICAL ONCOLOGY AND RADIOTHERAPY 20 (2015) 309–315

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/rpor

Case report

Hypersensitivity to chemoradiation in FANCA carrier with cervical carcinoma—A case report and review of the literature

Igor Sirák[a,*], *Zuzana Šinkorová*[b], *Mária Šenkeříková*[c], *Jiří Špaček*[d], *Jan Laco*[e], *Hana Vošmiková*[e], *Stanislav John*[a], *Jiří Petera*[a]

[a] *Department of Oncology and Radiotherapy, University Hospital Hradec Králové, Sokolská 581, Hradec Kralove 500 05, Czech Republic*

[b] *Department of Radiobiology, Faculty of Health Sciences, University of Defence in Brno, Sokolská 581, Hradec Kralove 500 05, Czech Republic*

[c] *Department of Medical Genetics, University Hospital Hradec Králové, Sokolská 581, Hradec Kralove 500 05, Czech Republic*

[d] *Department of Gynecology and Obstetrics, University Hospital Hradec Králové, Sokolská 581, Hradec Kralove 500 05, Czech Republic*

[e] *The Fingerland Department of Pathology, Charles University in Prague, Faculty of Medicine and University Hospital Hradec Králové, Sokolská 581, Hradec Kralove 500 05, Czech Republic*

ARTICLE INFO

Article history:
Received 8 August 2014
Received in revised form
1 October 2014
Accepted 17 November 2014

Keywords:
Fanconi anemia
FANCA carrier
Heterozygote
Radiotherapy
Cisplatin

ABSTRACT

Objective: Compared to Fanconi anemia (FA) patients with homozygous defective two-alleles inheritance, there is a scarce or no evidence on one defective allele FANCA carriers, with respect to their cancer incidence, clinical and in vitro radiosensitivity and chemosensitivity. On that account, we report a case of a 30-year old FANCA mutation carrier woman with uterine cervix adenocarcinoma who was treated with chemoradiotherapy, in which unexpected acute toxicity and fatal late morbidity occured.

Methods: We also report the results of an in vitro test for radiosensitivity, immunohistochemical examination with FANCA staining and human papillomavirus genotypization, and a review of the literature for FA carrier patients with respect to cancer incidence, clinical and in vitro response to chemo/radiotherapy, options of early heterozygosity detection, and methods of in vitro prediction of hypersensitivity to oncologic treatment.

Conclusion: Although there are no standard guidelines for management of FA carriers with malignancies and reports about chemo- or radiosensitivity in this population are scarce; patients with FA-A heterozygosity may have a high rate of complications from chemo/radiotherapy. Up to now, an optimum method for the prediction of radiosensitivity and the best parameter has not been found. Clinical radioresponsiveness is unpredictable in FA carriers and there is a pressing need of new rapid and predictive in vitro assays of

* *Corresponding author*. Tel.: +420 495 832 176; fax: +420 495 832 081.
E-mail address: igor.sirak@fnhk.cz (I. Sirák).

http://dx.doi.org/10.1016/j.rpor.2014.11.006

1507-1367/© 2014 Greater Poland Cancer Centre. Published by Elsevier Urban & Partner Sp. z o.o. All rights reserved.

radiation responses. Until then, the treatment of FA carriers with malignancies should be individualized, with respect to potential hypersensitivity to ionizing radiation or cross-linking agents.

© 2014 Greater Poland Cancer Centre. Published by Elsevier Urban & Partner Sp. z o.o. All rights reserved.

1. Introduction

Fanconi anemia (FA) is a rare autosomal recessive DNA repair disorder that causes chromosomal instability, cancer susceptibility, and radiation sensitivity. Two mutated alleles in FA genes are required to cause the disease. Fourteen FA corresponding genes have been identified (*FANC-A*, *B*, *C*, *D1/BRCA2*, *D2*, *E*, *F*, *G*, *I*, *J/BRIP1*, *L M*, *N/PALB2*, and *O/RAD51C*). FANCA is the most frequent complementation group representing approximately two-thirds of FA patients.[1] The number of pathogenic variants described for the *FANCA* gene is very high considering the relatively low number of patients.[2–7] The incidence of FA has been estimated at 1–5/million, with a carrier frequency of 1/150–300.[8,9]

Clinical findings in FA include aplastic anemia and congenital abnormalities, such as microcephaly, short stature, radial ray bone abnormalities, abnormal skin pigmentation and gonads, malformations of the kidney and heart; developmental delay and mental retardation. FA patients usually develop solid tumors, such as carcinoma of the esophagus, oropharynx, cervix and vulva, but they tend to die more often from hematologic malignancies, such as leukemia or bone marrow failure.[10–17] The relative risk of cancer in FA patients is at least 50-fold that of the general population.[18] The cause of development of cancer in FA is thought to be attributable to a defect in maintaining the genome integrity, leading to increased chromosomal instability with defective DNA repair mechanism.[19]

FA patients are hypersensitive to radiotherapy (RT) and cytotoxic agents, althought there has been a variable scale of radiosensitivity and chemosensitivity reported, from fulminant toxicities in lowest doses to excellent "high-dose" treatment toleration.[13,14,20–26] Abnormal toxicity to RT is caused by defective FA pathway in the recognition of DNA damage and defective repair of DNA damage by homologous recombination. Radiosensitivity may represent the very first manifestation of FA in adult patients.[12,27] In vitro, patients with FA are radiosensitive, when tested by colony survival assay or Comet assay; FA cells also exhibit hypersensitivity to chemotherapy and other DNA cross-linking agents such as cisplatin or mitomycin. Discrepancy between in vitro and in vivo sensitivity to RT has been reported, and may cause a problem in toxicity prediction.[28–31]

Compared to FA patients, there is a scarce or no evidence on one defective allele FANCA carriers, with respect to their cancer incidence, clinical and in vitro radiosensitivity and chemosensitivity. On that account, we report a case of a young FANCA mutation carrier woman with uterine cervix carcinoma who was treated with definitive chemoradiation, in which unexpected acute toxicity as well as late morbidity occured. We also report a review of the literature for FA carrier patients with respect to cancer incidence, clinical and in vitro response to chemo/radiotherapy, options of early heterozygosity detection, and methods of in vitro prediction of hypersensitivity to oncologic treatment.

2. Case report

A 30-year old woman with cervical adenocarcinoma was referred to our department in September 2011. Her medical history was otherwise negative, except for nicotinism, 3 childbirths and 2 miscarriages. Her father and two brothers of him had all died of lung carcinoma, all heavy smokers. Her mother was still alive, treated with early breast carcioma. At the time of diagnosis, her 2 sisters and 1 brother were yet healthy, according to the patient.

Cervical biopsy revealed grade 1 adenocarcinoma of the cervix, with incipient parametrial invasion on MRI, without lymphadenopathy or distant metastases. Initial blood count, leukocyte differential, and chemistry results were all in tolerant ranges, except mild thrombocytopenia (123.000/μL) with slightly increased mean platelet volume (13.1 fL).

Because of parametrial invasion, the patient was decided for definitive pelvic chemoradiotherapy. Planned course of treatment consisted of 45 Gy in 25 fractions pelvic IMRT with weekly cisplatin of 40 mg/m^2 in 5 cycles and subsequent uterovaginal high-dose rate brachytherapy 5 times 6 Gy to the point A. After 10.8 Gy of radiotherapy and 2 cycles of cisplatin the treatment had to be interrupted due to severe pancytopenia, with protracted grade 4 thrombocytopenia (nadir <10.000/μL) with the need of repeated platelet transfusions. Other signs of toxicity comprised grade 2 anemia, grade 3 neutropenia, complete alopecia, grade 2 diarrhea and nausea (CTCAE v4.0). The treatment interruption lasted for 2 weeks, while we excluded any error in RT or chemotherapy mistaking. Then, with cisplatin held, the whole course of RT and brachytherapy were completed with moderate toxicity and intensive supportive care. The question of abnormal acute toxicity remained unanswered.

Six months after completion of chemoradiation, partial regression of primary tumor was evaluated, but still there was a residual biopsy-proven tumor isolated to cervix. Residual disease was followed by regular examination until there was MRI confirmed local progression isolated to the cervix. Salvage radical hysterectomy was performed with intention of bladder and rectum preservation. The microscopic examination of hysterectomy specimen revealed clear-cell adenocarcinoma of uterine cervix (Figs. 1 and 2).

Two months after surgery, vesico-vaginal fistula with vaginal cuff dehiscence occured; treated with bilateral Bricker ureterostomy. Peroperatively, massive adhesions were found in the irradiated area corresponding clinically with chronic

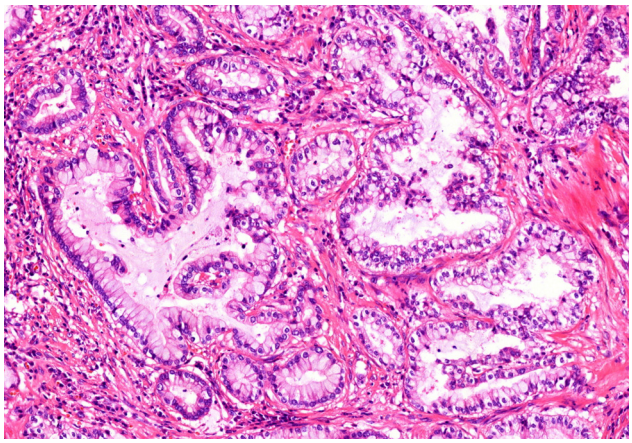

Fig. 1 – Tubulo-papillary arrangement of tumor cells (hematoxylin–eosin, original magnification 200×).

subileus, without clinical or pathogical signs of recurrence. Massive vaginal bleeding occured subsequently, with need of urgent endovascular internal illiac vessels embolization. One month later, jejuno-cutaneous fistula in right lower-abdomen region occured, treated by operative fistula resection with terminal jejunostomy. Peroperatively, coeco-vesico-vaginal fistula was diagnosed as well and terminated, again without peroperative evidence of cancer recurrence. Simultaneously came progression of cachexia, malnutrition, short bowel syndrome, immobility and depresion; necessitating permanent institutionalization, with parenteral alimentation and intensive medical and psychological support. She died of severe treatment consequences in September 2013, without evidence of disease progression.

2.1. *Molecular/genetic examination*

Until December 2012, there was no explanation both of acute toxicity and long-term treatment consequences available. At that time, her 17-year old brother was diagnosed with FA, manifested by progressive bone marrow failure

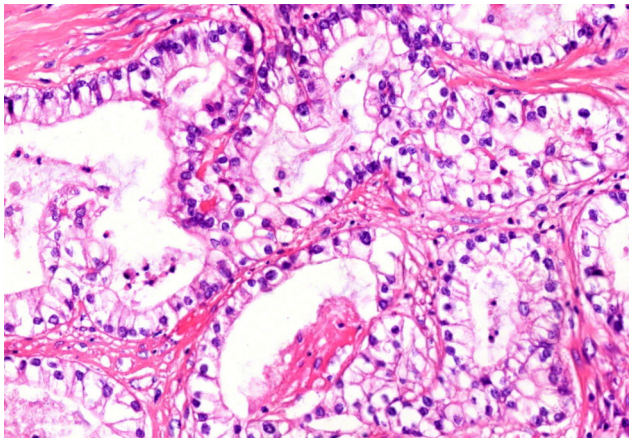

Fig. 2 – Polygonal tumor cells have enlarged nuclei and clear cytoplasm (hematoxylin–eosin, original magnification 400×).

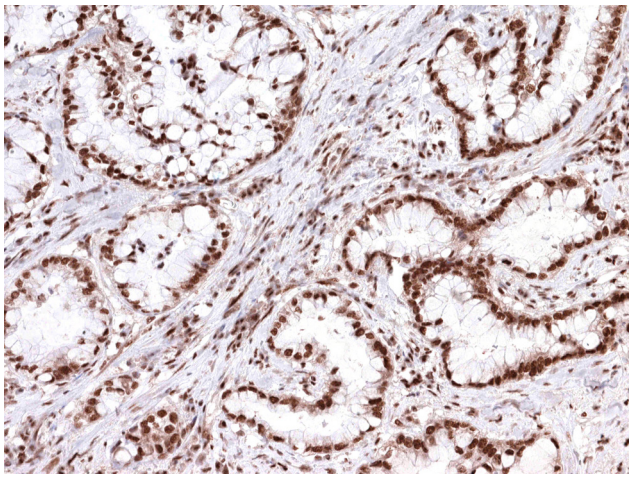

Fig. 3 – Strong nuclear expression of FANCA in tumor cells and in leukocytes and non-neoplastic fibroblasts of cervical stroma (original magnification 200×).

(with predominant thrombocytopenia); with characteristic microcephaly, hyperpigmentation, triphalangeal thumbs, reflux uropathy, mental retardation (since childhood), and ventricular septum defect. In him, molecular/genetic examination detected two causal FANCA mutations with composite heterozygosity for mutation c.3239G>T in exon 32 and c.3788_3790delTCT in exon 38. The laboratory diagnosis of FA was confirmed by functional exmanination of fibroblasts. On the grounds of these new findings, all family members including our patient were tested genetically. Molecular/genetic examination in our patient confirmed heterozygous mutation c.3788_3790delTCT in exon 38, proving her as a carrier of FANCA mutation. Following these results, in vitro testing of individual lymphocyte subpopulations radiosensitivity and immunohistochemical examination of hysterectomy material with focus on FA was performed; as well as genotypization for presence of human papillomavirus (HPV) deoxyribonucleic acid (DNA).

2.2. *Immunohistochemical examination*

The tissue specimens were routinely processed, embedded in paraffin and stained with hematoxylin eosin. Immunohistochemically, tumor cells showed diffuse expression of CK7, ARID1A and HNF-1β. CEA was positive in isolated tumor cells. Expression of CK20, vimentin, ER, PR, p16, p53 and WT-1 was absent. Diffuse expression of FANCA was observed in both tumor cells and non-neoplastic cervical tissue of our patient (Fig. 3); which represents the products of "the healthy FA-A allele". As a control for FANCA staining, we examined a trepanobiopsy specimen of her brother, which was negative in all bone marrow, fatty and muscle tissue, showing no production of FANCA protein at all (Fig. 4).

2.3. *HPV genotypization*

The Genotyping kit HPV GP (Diassay) was used for HPV polymerase chain reaction (PCR) genotypization according

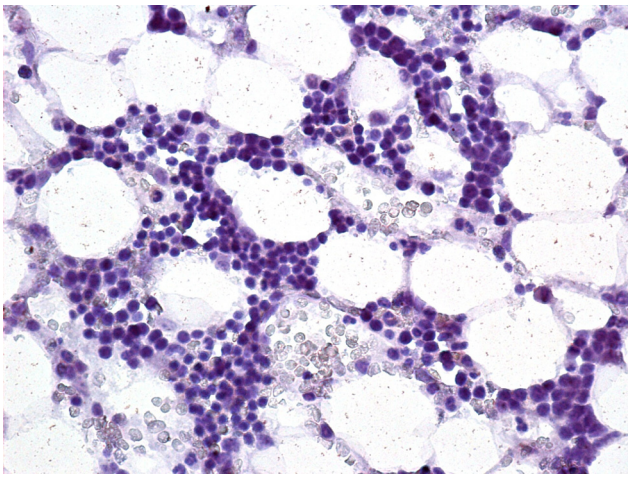

Fig. 4 – Absent FANCA expression in bone marrow cells in trephine biopsy of the patient's brother (original magnification 400×).

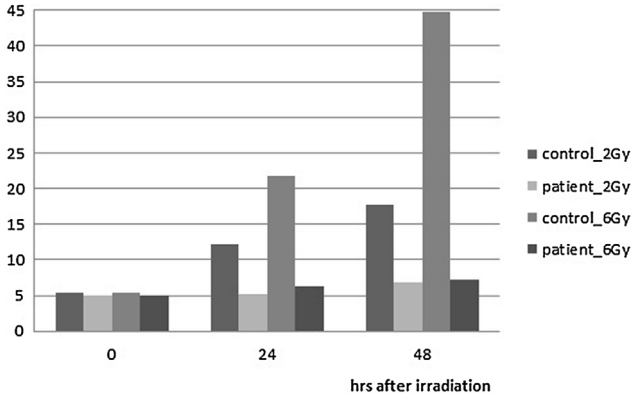

Fig. 5 – T-cell IVNIR/B-cell IVNIR in PBMC of the patient and control individual upon irradiation (2 and 6 Gy) and subsequent cultivation (24 and 48 h). The control sample behaved in a typical way when more radiosensitive B-cells decreased with dose and time of cultivation while patient's CD19+ B-cells were as radioresistant as their CD3+ counterparts.

to manufacturer's protocol. The kit is an in vitro reverse hybridization strip assay using GP5+/6+ primers for the qualitative identification of the individual high risk types HPV16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82 in DNA extracted from clinical specimens. The beta-globin primers were provided to control for sample quality and the presence of inhibiting factors. As a result, we did not prove any presence of HPV-DNA; high-risk nor low-risk. This corresponds with negative p16 staining mentioned above.

2.4. *In vitro radiosensitivity examination*

Heparin-treated blood obtained from the patient and a control healthy volunteer was used for separation of peripheral blood mononuclear cells (PBMC) by centrifugation. Individual plates were sham-treated or irradiated by doses of 2 or 6 Gy of ^{60}Co with a rate of 1.29 Gy/min at a distance of 1.23 m. The plates were then cultivated for 24 and 48 h at 37 °C; then harvested and centrifuged. Fluorochrome conjugated, human antigen-specific mouse monoclonal antibodies were used for multicolor immunophenotyping of PBMC in two cocktails. To eliminate differences in between individual blood donors, we have used the "irradiated versus non-irradiated ratio" (IVNIR) values as described elsewhere.[32] Different radiosensitivity of individual lymphocyte subpopulations and subsets were found between patient's and control PBMC in vitro. Upon irradiation and subsequent cultivation, the most radiosensitive population of CD19+ B cells exerted a typical time- and dose-dependent decrease in the control sample, while patient's B cells appeared much more radioresistant, which was reflected by an invariant T/B IVNIR ratio (Fig. 5). In parallel, the most radiosensitive CD27+ subset of B cells did not decrease in a typical manner in the patient PBMC. CD3+ T cells and their CD4+, CD8+, Treg (CD4+ CD25hi+) and CD27+ subsets had similar radiosensitivity both in the patient and control individual.

3. Review of the literature

Although obligate heterozygous individuals from FA families have not been extensively researched, evidence suggests that these are free from major clinical symptoms and have a normal life expectancy.[33] Studies of cancer incidence in FA relatives have produced conflicting results. The largest study of FA families has not shown any difference in overall cancer risk in FA relatives.[34] Similarly, another registry study showed no suggestion of an increase in cancer incidence among FA relatives.[35] These are important studies for FA carriers, with correspondence to anticipated hypersensitivity to oncologic treatment.

Although severe adverse reactions to RT are rare, they are usually associated with individuals with DNA repair disorders. As many as 40 DNA repair defective disorders exist that are associated with radiosensitivity or sensitivity to DNA damaging agents used to treat cancer.[26] Many reports have been published to detail such circimstances. FA is associated with more than one-half of these reports; while most reported FA patients died within months of exposure.[24] The lowest radiation dose associated with a fatal outcome in FA patient reported is 8 Gy.[21] In these reports, radiosensitivity was associated closely with the homozygous inheritance of defective proteins necessary for recognition and/or repair of DNA double strand breaks.[36] Radiosensitive individuals represent the greatest potential risk of accidental injury; although this includes a small number of primarily homozygotes with DNA repair disorders, it also includes a much greater number of likely heterozygotes for these same disorders, albeit at a greater treshold of radiosensitivity.

Evidence on FA carriers with respect to chemo/radiotherapy is limited. This may be caused particulary by underdiagnosis of FA heterozygosity in common patient population. One defective FA allele may cause abnormal reactions (with variable severity) in 1 of circa 300 patients treated worldwide

on the regullar basis; however this is usually unrecognized, as other factors play role in individual treatment tolerance. For physician, it is entirely impossible to recognize FA carriers during the first contact with the patient, unless positive FA history in the family is present; however this would be rare situation (probability < 1:1000 depending on number of generations and family subjects availible), according to FA prevalence.

Rapid methods for quickly screening patients before RT are not yet validated for clinical use. Colony survival assays can yield definitive results in 12 weeks, providing some advance warning of radiosensitivity in homozygous patients.[37] However, hegterozygous carriers are not clearly identified by this method. The laboratory identification of heterozygosity for DNA repair disorders is in its infancy;[38] thus it has been difficult to search for correlations with clinical radiosensitivity. More sensitive and reliable assays are needed to identify this large segment of patients, which probably includes as much as 5% of the population.[39]

Other in vitro tests of radiosensitivity to Colony survival assay may be helpful to recognize heterozygous carriers. E.g., cells of both FA homozygous and heterozygous patients exposed in vitro to X-radiation showed uniformly high initial DNA damage rates as assesed by the single-cell gell comet assay.[40] Morover, the repair half-time parameters were significantly elevated both in FA patients and carriers in this study which concluded, that comet assay may be a useful adjunct for heterozygote detection in families of FA patients. Similarly, in the study of Mohseni, in vitro radisensitivity in FA patients and heterozygotes was assessed using alkaline comet assay. In their study, the amount of residual DNA damage after irradiation could be used as a putative predictor of FA screening and cellular radiosensitivity.[41] Other less frequent experimental techniques have been adopted to correlate the in vitro radiation sensitivity with the outcome of therapy.[42] Western blot and foci immunofluorescence analysis give qualitative results, but lack correlation between in vitro results and in vivo radiation response.[43] In view of the conflicting data on radiosensitivity of FA cells, there is a pressing need for the development of new rapid and predictive assays of radiation responses.

We and others have previouly shown that B-cells are the most radiosensitive lymhocyte population both in vitro and in vivo,[43,44] while apparent radiosensitivity of NK cells strongly differs under in vivo and in vitro conditions where this population of phylogenetically oldest lymhocytes exerts the lowest and higest reduction in relative numbers, respectively.[45,46] We have documented B-cells from our patient to be much more radioresistant than their counterparts in healthy individual in vitro. While control B-cells and their subsets characterized by the exression of CD21, CD27 and CD38 surface markers behaved as was expected, the usual depletion of B-cells and their subsets did not occur in the FA carrier. This finding indcates that irradiation-induced programmed cell death is impaired in the FA heterozygous individuals, which may affect the efficiency of the radiotherapy.

Data about in vitro hypersensitivity of FA heterozygotes to cytotoxic agents are scarce and controversial. Some experiments reported, that FA heterozygotes do not show an increased chromosomal sensitivity to chemotherapeuticals and thus cannot be differentiated from the normal population.[47,48] Auerbach et al.[49] were the first to claim that it was possible to distinguish between controls and heterozygotes by using the diepoxybutane (DEB). Lately Marx et al. detected an increase in DEB sensitivity of lymphocytes of obligate carriers sufficient enough to differentiate them from controls.[50] Recently it has been recognized that the DEB test can give false-negative results in patients with somatic mosaicism[51]; moreover, the DEB test cannot be used reliably to distinguish between FA carriers and normal individuals. Mohseni et al. reported slightly higher induced aberrations by mitomicin-C (MMC) for obligate FA carriers than for controls. However, the difference was not statistically significant enough to allow accurate distinction between the two groups.[52] Another study of in vitro exposure of cells to bleomycin proved significant difference in induced chromatid breaks between three FA heterozygotes and 11 controls.[53] This study supports our clinical observation of abnormal toxicity after cisplatin-based chemoradiation in our patient, with convincing hypersensitivity to cisplatin as complete alopecia could not be caused by RT alone.

Patients with FA who survive their adolescency typically develop solid squamous cell carcinomas (SCC) of the head-and-neck or the female genital tract.[18,54,55] These malignancies are commonly associated with HPV infection, and are often multifocal in distribution. There are some reports on young FA patients with multiple HPV-associated SCC published[56]; indicating possible relationship between HPV-associated malignancies and FA mutations.[57,58] In 2009, Wang et al. reported some important findings, that also FANCA variants (in addition to FA mutations) may be an important host event involved in cervical carcinoma susceptibility, but not HPV infection persistence.[59] Likewise in our patient, adenocarcinoma of the cervix developed at the age of 30, with negative p16 staining and negative HPV-DNA PCR genotypization; showing no association with HPV infection in this case. This manuscript represents a piece of our investigation into cervical carcinoma genetics and prediction of response to chemo/radiation.[60–64]

4. Conclusions

Although there are no standard guidelines for management of FA carriers with malignancies and reports about chemo- or radiosensitivity in this population are scarce; patients with FA-A heterozygosity may have a high rate of complications from chemoradiotherapy. Up to now, an optimum method for the prediction of radiosensitivity and the best parameter has not been found. Clinical radioresponsiveness is unpredictable in FA carriers and there is a pressing need of new rapid and predictive in vitro assays of radiation responses. Until then, the treatment of FA carriers with malignancies should be individualized, with respect to potential hypersensitivity to ionizing radiation or cross-linking agents.

Conflict of interest

None declared.

Financial dislosure

None declared.

Acknowledgements

Supported by the program PRVOUK P37/06; PRVOUK P37/11; project LM2010004; and Research project NT11334-4/2010 of Ministry of Health, Czech Republic.

REFERENCES

1. Castella M, Pujol R, Callén E, et al. Origin, functional role, and clinical impact of Fanconi anemia FANCA mutations. *Blood* 2011;**117**:3759–69.
2. Wijker M, Morgan NV, Herterich S, et al. Heterogeneous spectrum of mutations in the Fanconi anaemia group A gene. *Eur J Hum Genet* 1999;**7**:52–9.
3. Demuth I, Wlodarski M, Tipping AJ, et al. Spectrum of mutations in the Fanconi anaemia group G gene, FANCG/XRCC9. *Eur J Hum Genet* 2000;**8**:861–8.
4. Bouchlaka C, Abdelhak S, Amouri A, et al. Fanconi anemia in Tunisia: high prevalence of group A and identification of new FANCA mutations. *J Hum Genet* 2003;**48**:352–61.
5. Levran O, Diotti R, Pujara K, et al. Spectrum of sequence variations in the FANCA gene: an International Fanconi Anemia Registry (IFAR) study. *Hum Mutat* 2005;**25**:142–9.
6. Savino M, Borriello A, D'Apolito M, et al. Spectrum of FANCA mutations in Italian Fanconi anemia patients: identification of six novel alleles and phenotypic characterization of the S858R variant. *Hum Mutat* 2003;**22**:338–9.
7. Yagasaki H, Hamanoue S, Oda T, et al. Identification and characterization of novel mutations of the major Fanconi anemia gene FANCA in the Japanese population. *Hum Mutat* 2004;**24**:481–90.
8. Rosenberg PS, Tamary H, Alter BP. How high are carrier frequencies of rare recessive syndromes? Contemporary estimates for Fanconi anemia in the United States and Israel. *Am J Med Genet* 2011;**155A**:1877–83.
9. Jacquemont C, Toniguchi T. The Fanconi anemia pathway and ubiquitin. *Biochem* 2007;**8**(Suppl. 1):S1–10.
10. Budrukkar A, Shahid T, Murthy V, et al. Squamous cell carcinoma of base of tongue in a patient with Fanconi's anemia treated with radiation therapy: case report and review of literature. *Head Neck* 2010;**32**:1422–7.
11. Carvalho JP, Dias ML, Carvalho FM, et al. Squamous cell vulvar carcinoma associated with Fanconi's anemia: a case report. *Int J Gynecol Cancer* 2002;**12**:220–2.
12. Kutler DI, Auerbach AD, Singh B, et al. High incidence of head and neck squamous cell carcinoma in patients with Fanconi anemia. *Arch Otolaryngol Head Neck Surg* 2003;**129**:106–12.
13. Harper JL, Jenrette JM, Goddu SM, et al. Vulvar cancer in a patient with Fanconi's anemia, treated with 3D conformal radiotherapy. *Am J Hematol* 2004;**76**:148–51.
14. Hosoya Y, Lefor A, Hirashima Y, et al. Successful treatment of esophageal squamous cell carcinoma in a patient with Fanconi anemia. *Jpn J Clin Oncol* 2010;**40**:805–10.
15. Masserot C, Peffault de Latour R, Rocha V, et al. Head and neck squamous cell carcinoma in 13 patients with Fanconi anemia after hematopoietic stem cell transplantation. *Cancer* 2008;**113**:3315–22.
16. Scheckenbach K, Wagenmann M, Freund M, et al. Squamous cell carcinomas of the head and neck in Fanconi anemia: risk, prevention, therapy, and the need for guidelines. *Klin Padiatr* 2012;**224**:132–8.
17. Mousavi A, Abbasi F, Abadi AG, Hashemi FA. Vulvar squamous cell carcinoma associated with Fanconi's anemia. *Int J Hematol* 2010;**91**:498–500.
18. Alter BP, Greene MH, Velazquez I, et al. Cancer in Fanconi anemia. *Blood* 2003;**101**:2072.
19. Rosenberg PS, Greene MH, Alter BP. Cancer incidence in persons with Fanconi's anemia. *Blood* 2003;**101**:822–6.
20. Alter BP. Radiosensitivity in Fanconi's anemia patients. *Radiother Oncol* 2002;**62**:345–7.
21. Bremer M, Schinder D, Gross M, et al. Fanconi's anemia and clinical radiosensitivity report on two adult patients with locally advanced solid tumors treated by radiotherapy. *Strahlenter Onkol* 2003;**179**:748–53.
22. Marcou Y, D'Andrea A, Jeggo PA, Plowman PN. Normal cellular radiosensitivity in an adult Fanconi anemia patient with marked clinical radiosensitivity. *Radiother Oncol* 2001;**60**: 75–9.
23. Tan IB, Cutcutache I, Zang ZJ, et al. Fanconi's anemia in adulthood: chemoradiation-induced bone marrow failure and a novel FANCA mutation identified by targeted deep sequencing. *J Clin Oncol* 2011;**29**:e591–4.
24. Birkeland AC, Auerbach AD, Sanborn E, et al. Postoperative clinical radiosensitivity in patients with fanconi anemia and head and neck squamous cell carcinoma. *Arch Otolaryngol Head Neck Surg* 2011;**137**:930–4.
25. Spanier G, Pohl F, Giese T, et al. Fatal course of tonsillar squamous cell carcinoma associated with Fanconi anaemia: a mini review. *J Craniomaxillofac Surg* 2012;**40**:510–5.
26. Pollard JM, Gatti RA. Clinical radiation sensitivity with DNA repair disorders: an overview. *Int J Radiat Oncol Biol Phys* 2009;**74**:1323–31.
27. Huck K, Hanenberg H, Gudowius S, et al. Delayed diagnosis and complications of Fanconi anaemia at advanced age – a paradigm. *Br J Haematol* 2006;**133**:188–97.
28. Rogers PB, Plowman PN, Harris SJ, Arlett CF. Four radiation hypersensitivity cases and their implications for clinical radiotherapy. *Radiother Oncol* 2000;**57**:143–54.
29. Djuzenova C, Flentje M, Plowman PN. Radiation response in vitro of fibroblasts from a fanconi anemia patient with marked clinical radiosensitivity. *Strahlenther Oncol* 2004;**180**:789–97.
30. Burnet NG, Peacock JH. Normal cellular radiosensitivity in an adult Fanconi anaemia patient with marked clinical radiosensitivity. *Radiother Oncol* 2002;**62**:350–1.
31. Carreau M, Alon N, Bosnoyan-Collins L, et al. Drug sensitivity spectra in Fanconi's anemia lymphoblastoid cell lines of defined complementation groups. *Mutat Res* 1999;**435**:103–9.
32. Rehakova Z, Sinkora J, Vlkova M, et al. CD27(+) peripheral blood B-cells are a useful biodosimetric marker in vitro. *Physiol Res* 2008;**57**:589–600.
33. Heim RA, Lench NJ, Swift M. Heterozygous manifestations in four utosomal recessive human cancer prone syndromes: ataxia telangiectasia, xeroderma pigmentosum, Fanconi anemia, and Bloom syndrome. *Mutat Res* 1992;**284**:25–36.
34. Tischkowitz M, Easton DF, Ball J, et al. Cancer incidence in relatives of British Fanconi anaemia patients. *BMC Cancer* 2008;**8**:257.
35. Berwick M, Satagopan JM, Ben-Porat L, et al. Genetic heterogeneity among Fanconi anemia heterozygotes and risk of cancer. *Cancer Res* 2007;**67**:9591–6.
36. Gatti RA. The inherited basis of human radiosensitivity. *Acta Oncol* 2001;**40**:702–11.
37. Huo YK, Wang Z, Hong JH, et al. Radiosensitivity of ataxia-teleangiectasia, X-linked agammaglobulinemia and related syndromes using a modified colony survival assay. *Cancer Res* 1994;**54**:2544–7.

38. Nahas S, Butch A, Gatti RA. Rapid flow cytometry-based SMC1 phophorylation assay for identification of ataxia-teleangiectasia homozygotes and heterozygotes. *Clin Chem* 2009;**55**:463–72.
39. Leong T, Chamo M, Bassal S, et al. Radiation-hypersensitive cancer patients do not manifest protein expression abnormalities in components of the nonhomologous end-joining (NHEJ) pathway. *Br J Cancer* 2003;**88**: 1251–5.
40. Djuzenova CS, Rothfuss A, Oppitz U, et al. Response to X-radiation of fanconi anemia homozygous and heterozygous cells asseses by the single-cell gel electrophoresis (comet) assay. *Lab Invest* 2001;**81**: 185–92.
41. Mohseni-Meybodi A, Mozdarani H, Mozdarani S. DNA damage and repair of leukocytes from Fanconi anaemia patients, carriers and healthy individuals as measured by the alkaline comet assay. *Mutagenesis* 2009;**24**:67–73.
42. Arlett CF, Harcourt SA. Survey of radiosensitivity in a variety of human cell strains. *Cancer Res* 1980;**40**:926–32.
43. Louagie H, Van Eijkeren M, Philippé J, et al. Changes in peripheral blood lymphocyte subsets in patients undergoing radiotherapy. *Int J Radiat Biol* 1999;**75**:767–71.
44. Sinkorova Z, Zarybnicka L, Matoskova L, et al. Dynamics of apoptosis in γ-irradiated lymphocytes and the CD21+ CD27+ B-cell subset as a potential biodosimetric marker. *Radiat Meas* 2011;**46**:1009–13.
45. Vokurková D, Sinkora J, Vávrová J, et al. CD8+ natural killer cells have a potential of a sensitive and reliable biodosimetric marker in vitro. *Physiol Res* 2006;**55**:689–98.
46. Philippé J, Louagie H, Thierens H, et al. Quantification of apoptosis in lymphocyte subsets and effect of apoptosis on apparent expression of membrane antigens. *Cytometry* 1997;**29**:242–9.
47. Cohen MM, Simpson SJ, Honig GR, et al. The identification of Fanconi anemia genotypes by clastogen stress. *Am J Hum Genet* 1982;**34**:794–810.
48. Wu X, Gu J, Hong WK, et al. Benzo[a]pyrene diol epoxide and bleomycin sensitivity and susceptibility to cancer of upper aero digestive tract. *J Natl Cancer Inst* 1998;**90**:1393–9.
49. Auerbach AD, Warburton D, Bloom AD, Chaganti RSK. Prenatal detection of the Fanconi anemia gene by cytogenetic methods. *Am J Hum Genet* 1979;**31**:77–81.
50. Marx MP, Smith S, Heyns AD, van Tonder IZ. Fanconi's anemia: a cytogenetic study on lymphocyte and bone marrow cultures utilizing 1,2:3,4-diepoxybutane. *Cancer Genet Cytogenet* 1983;**9**:51–60.
51. Dokal I, Chase A, Morgan NV, et al. Positive diepoxybutane test in only one of two brothers found to be compound heterozygotes for Fanconi's anaemia complementation group C mutations. *Br J Haematol* 1996;**3**:813–6.
52. Mohseni-Meybodi A, Mozdarani H, Vosough P. Cytogenetic sensitivity of G0 lymphocytes of Fanconi anemia patients and obligate carriers to mitomycin C and ionizing radiation. *Cytogenet Genome Res* 2007;**119**:191–5.
53. Barquinero JF, Barrios L, Ribas M, et al. Cytogenetic sensitivity of three Fanconi anemia heterozygotes to bleomycin and ionizing radiation. *Cancer Genet Cytogenet* 2001;**124**:80–3.
54. Kutler DI, Wreesmann VB, Goberdhan A, et al. Human papillomavirus DNA and p53 polymorphisms in squamous cell carcinomas from Fanconi anemia patients. *J Natl Cancer Inst* 2003;**95**:1718–21.
55. Kutler DI, Singh B, Satagopan J, et al. A 20-year perspective on the International Fanconi Anemia Registry (IFAR). *Blood* 2003;**101**:1249–56.
56. Han TJ, Lee CH, Yoo CW, et al. Synchronous multifocal HPV-related neoplasm involving both the genital tract and the head-and-neck area: a case report of Fanconi anemia. *Radiother Oncol* 2009;**92**:138–41.
57. Spardy N, Duensing A, Charles D, et al. The human papillomavirus type 16 E7 oncoprotein activates the Fanconi anemia (FA) pathway and causes accelerated chromosomal instability in FA cells. *J Virol* 2007;**81**:13265–70.
58. Hoskins EE, Morris TA, Higginbotham JM, et al. Fanconi anemia deficiency stimulates HPV-associated hyperplastic growth in organotypic epithelial raft culture. *Oncogene* 2009;**28**:674–85.
59. Wang SS, Bratti C, Rodriguez AC, et al. Variants in immune and DNA repair genes and risk for human papillomavirus persistence and progression to cervical cancer. *J Infect Dis* 2009;**199**:20–30.
60. Sirak I, Petera J, Zoul Z. The influence of cisplatin dose upon survival in concurrent chemoradiotherapy of locallyadvanced cervical carcinoma with weekly cisplatin. *Acta Medica (Hradec Kralove)* 2008;**51**:95–9.
61. Petera J, Sirak I, Beranek M, et al. Molecular predictive factors of outcome of radiotherapy in cervical cancer. *Neoplasma* 2011;**58**:469–75.
62. Beranek M, Drastikova M, Paulikova S, Sirak I, Vosmik M, Petera J. Analysis of D1853N ATM polymorphism in radio sensitive patients with cervical carcinoma. *Acta Medica (Hradec Kralove)* 2011;**54**:111–6.
63. Vosmik M, Laco J, Sirak I, et al. Prognostic significance of human papillomavirus (HPV) status and expression of selected markers (HER2/neu, EGFR, VEGF, CD34, p63, p53 and Ki67/MIB-1) on outcome after (Chemo-) radiotherapy in patients with squamous cell carcinoma of uterine cervix. *Pathol Oncol Res* 2014;**20**:131–7.
64. Paulikova S, Petera J, Sirak I, et al. ATM and TGFB1 genes polymorphisms in prediction of late complications of chemoradiotherapy in patients with locally advanced cervical cancer. *Neoplasma* 2014;**61**:70–6.