Induction of Apoptosis and Identification of Apoptosis-related Genetic Susceptibility in Response to Radiation in Murine Tumors

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1. Introduction

Radiation-induced apoptosis varies among different tumors but it positively correlates with the anti-tumor efficacy of radiation [1], which makes apoptosis a potential predictor of tumor treatment outcome after radiotherapy [2,3]. Moreover the regulation of apoptosis induction may be used for the improvement of radiotherapy through either increasing apoptotic response of normal tissues.The p53 has a beneficial role in tumor therapy was recently demonstrated by transferring wild-type p53 to cells of tumor xenografts or to tumors in humans.

Also, proteomics approache to the identification of novel biomarkers for cancer diagnosis have traditionally relied on the identification of differentially expressed proteins tumor cells based on the patterns of protein expression observed by two-dimensional gel electrophoresis .

2. Methods and Results

In this study, two murine carcinomas synenetic to C3H/HeJ were used : OCa-I, the ovarian carcinomas and HCa-I, the hepatocarcinoma. The two tumors have been reported to show the same wild type p53 [4,5], but distinctly different radiosensitivities. In OCa-I and HCa-I, the specific growth delays were 12.7days and 0.3days and TCD50s were 52.6 Gy and more than 80 Gy, respectively.

Tumors, 8 mm in diameter, were irradiated with 25 Gy and at various times after irradiation, ranging from 1 to 48h, were analyzed histologically for apoptosis.

To identify radio-genetic susceptibilitic protein in HCa-I and OCa-I tumors was analyzed for their two-dimensional electrophoresis (2DE) and matrix-assisted spectrometry (MALDI-TOF MS) [6].

2.1 Analysis of Apoptosis

Figure.1 shows apoptosis induction in OCa-I and HCa-I tumors as a function of time, ranging from 1 to 48h, after 25 Gy tumor irradiation. Untreated OCa-I tumors contained 1.8 ± 0.6% and HCa-I tumor 0.2 ± 0.0% apoptotic cells. Radiation rapidly induced apoptosis in OCa-I which peaked already at 2h after the treatment at which time 16.1±0.6% cells were apoptotic.

After this the percentage of apoptotic cells declined, remained elevated 1 day later and approached the background level 2 days after irradiation. In contrast to its effect on OCa-I, radiation was totally ineffective in inducing apoptosis in HCa-I, findings similar to those of our earlier researches. The insert in Figure.1 shows that after 25Gy the apoptosis sensitive OCa-I exhibited rapid partial regression and long growth delayed, whereas the apoptosis resistant Hca-I showed only a small tumor growth delay that began several days after irradiation.

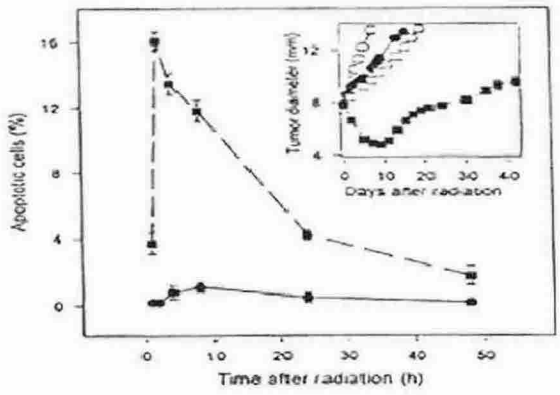

Figure 1. Induction of apoptosis in OCa-I (squares) and HCa-I (circles) murine tumors as a function of time following 25 Gy local tumor irradiation. Tumors were 8 mm at the time of irradiation. Vertical bars, SE of the mean. In the insert are growth curves of OCa-I (solid symbols) and HCa- I (open symbols), untreated (circles) or treated with 25 Gy (squares).

2.2 Proteomic analysis of the irradiated OCa-I proteins

To study the different proteins involved in ionizing radiations, we applied 2DE to analyze the proteomics alteration of wild-type p53 murine tumors. Image analysis of 2-DE gels reveals that averages of 800 proteins spots were detected and localized in pI 3-10 and molecular mass range 10-100 kDa. Comparison of these relatively abundant proteins on 2-DE gels using PDQUEST program revealed that protein spots were changed with their expression levels more than three fold in irradiated tissue. Those proteins were identified by peptide mass fingerprinting using MALDI-TOF mass spectrometry. Searching the MS-Fit database identified the peptide mass data.

10 proteins were changed at radiation in OCa-I. These include Sorting nexin 12, Elk protein, seven transmembrane helix receptor, zinc-binding protein 1, Protein phosphatase-1 regulatory subunit 7 beta 1, etc. But 3 proteins such as cytochrom c oxidase, mitogen activated protein kinase p38-2, myogenic factor 5 were changed significantly in the irradiated OCa-I (Table 1). In irradiated, OCa-I proteins were categorized listed of apoptosis, signal transduction, angiogenesis, cell cycle,

DNA repair, cytokine. The expression of cytochrom c oxidase protein increased in irradiated OCa-I.

Table.1 Identification of proteins differentially expressed in radiation-treated HCa-I and OCa-I

Type of tumor	Name of protein	Pattern change	Accession Number	M.W./P.I.	Function
HCa-I	Calpastain	▼	2765346	18,716/4.8	apoptosis
	Mitogen-activated protein kinase kinase	▼	1082585	36,172/6.2	signal transduction
OCa-I	Cytochrom C oxidase	▲	P14854	10,192/6.5	apoptosis
	Mitogen activated protein kinase p38-2	▼	387326	41,358/5.6	signal transduction
	Myogenic factor 5	▲	27806531	28,242/5.7	signal transduction

▲: increase ▼:decrease

2.3 Proteomic analysis of the irradiated HCa-I proteins

In irradiated, HCa-I proteins were categorized listed of apoptosis and signal transduction. Especially 2 proteins such as calpastain , mitogen-activated protein kinase kinase were changed significantly in the irradiated HCa-I (Table 1). In HCa-I radiation decreased the expression of both calpastain and mitogen-activated protein kinase kinase.

MAPK pathway has been implicated as mediators of apoptosis in radioresponse [7]. These results showed that the radiation-sensitive OCa-I decreased Mitogen activated protein kinase p38-2 and the radiation-resistant HCa-I decreased Mitogen-activated protein kinase kinase.

3. Conclusion

In conclusion, the results suggest that murine tumors possessing wild-type p53 can be sensitive or resistant to apoptosis induction by radiation, which in turn manifested in greater or poorer tumor growth delay (Figure.1) or tumor radiocurability. Differential expression analysis of proteomes may be useful for the development of new molecular markers for diagnosis and prognosis ofcarcinoma.

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