Pathology – Research and Practice 213 (2017) 1231–1234

Contents lists available at ScienceDirect

Pathology – Research and Practice

journal homepage: www.elsevier.com/locate/prp

Original article

Morphology based scoring of chromosomal instability and its correlation with cell viability

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ARTICLE INFO

Article history:
Received 29 December 2016
Received in revised form 30 May 2017
Accepted 24 June 2017

Keywords:
Chromosomal instability
Micronucleus
Nuclear buds
Aneuploidy
Scoring

ABSTRACT

Objective: The aim of this study was to devise the quantitative scoring system for Chromosomal instability (CIN) based on morphological indicators like MPM, NB, NPB, CS, La and MN in cancer cell line and to correlate it with cell viability and death.
Methods: Human hepatocellular carcinoma (HepG2) cells were treated with drugs like Diethylstilbestrol 0–100 μM, Griseofulvin 0–40 μg/ml, Vincristine sulphate 0–25 μg/ml, Mitomycin C 0–600 ng/ml, Bleomycin 0–10 μg/ml, Doxorubicin 0–30 μg/ml for 24 h. Following this, the CIN was assessed by counting the morphological indicators like Micronuclei (MN), Nuclear Buds (NB), Nucleoplasmic bridges, Laggards, Multipolar mitosis and chromatin strings/1000 cells in Giemsa stained smears by light microscopy and by determining the percentage of aneuploid cells by flow cytometry. The cell viability was assessed by MTT assay and percentage of apoptotic cells was determined by flow cytometry.
Results: The MN and NB were most frequently seen indicators and main determinants of morphological CIN. However, the morphological CIN score did not show any correlation with cell viability and apoptosis. Aneuploidy however was found to correlate positively with cell viability and NB score in our study (P-value <0.05).
Conclusions: The study for the 1st time attempted to develop a scoring system for CIN based on morphological parameters. However, a no correlation was observed between the later and cell viability or apoptosis. More robust techniques to quantify CIN may perhaps be more helpful in exploring the true link between CIN and cell viability in future.

1. Introduction

Cancers are characterized by genetic and chromosomal instability (CIN). The CIN occurring in tumours consist of abnormalities pertaining to chromosome numbers and structure [1]. Several mechanisms lead to CIN in cancer cells, including the defects in chromosome segregation, in mitotic checkpoints that guard against reproduction of abnormal cells, unstable telomeres and defective DNA damage response. CIN not only plays a role in initiation, progression and spread of cancer but is also believed to be a common cause of resistance of tumour cells to therapy [2]. Morphologically, CIN can be detected using various indicators such as presence of multipolar mitosis (MPM), nuclear buds (NB), micronuclei (MN), nucleoplasmic bridge (NPB), chromatin strings (CS) and laggards (La) [3]. These indicators are however is not routinely assessed quantitatively in the diagnostic setting. The aim of this study is to devise the quantitative scoring system for CIN based on morphological indicators like MPM, NB, NPB, CS, La and MN in cancer cell line and to correlate the same with tumour cell viability and apoptosis.

2. Materials and methods

Human hepatocellular carcinoma cell line (HepG2) used in the study was procured from National Centre for Cell Sciences (Pune, India). The CIN was induced by treatment with aneugenic (Diethylstilbestrol 0–100 μM, Griseofulvin 0–40 μg/ml, Vincristine sulphate 0–25 μg/ml) and clastogenic (Mitomycin C 0–600 ng/ml, Bleomycin 0–10 μg/ml, Doxorubicin 0–30 μg/ml) drugs for 24 h. Following this, the CIN was assessed by counting the above mentioned morphological indicators/1000 cells in Giemsa stained smears by light microscopy and by determining the percentage of aneuploid cells by flow cytometry. Total morphological CIN (CINt) was calculated by adding the counted number each of the markers/1000cells. Arbitrarily, the morphological CIN (CINs) was scored as: Score1: 1–60, Score 2: 61–120, Score 3: > 120.The cell viability

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http://dx.doi.org/10.1016/j.prp.2017.06.015
0344-0338/

Table 1
Table showing Mean cell viability, apoptosis, aneuploidy, MN, NB count, total morphological CIN and CIN score in HepG2 cells treated with different drugs.

Dose	Viability (%)	Apoptosis (%)	Aneuploidy (%)	MN/1000	NB/1000	CINt/1000	CINs
No Drug	100	1.1	47.74	32	23	60	1
Diethylstilbestrol(μM)							
20	76.57	2.1	50.64	5	8	13	1
40	69.35	3.6	70.17	57	2	59	1
60	64.7	4.4	81.86	8	7	15	1
100	46	31.8	68.54	2	31	33	1
Griseofulvin(μg)							
10	120.9	1.1	37.65	24	14	50	1
20	122.1	0.6	67.35	37	23	70	2
30	108.84	1.9	40.97	46	23	83	2
40	132.16	1.2	30.01	46	22	74	2
Vincristine (μg)							
5	105.02	16.7	37.66	24	18	42	1
10	92.57	10.8	43.35	21	17	38	1
15	103.62	15	15.65	20	13	33	1
25	99.97	2.5	11.89	17	16	33	1
Mitomycin C (ng)							
100	92.81	5.5	50.56	41	35	97	2
200	87.61	6.5	53.18	46	32	93	2
400	87.78	8.2	65.87	65	31	107	2
600	107.36	4.3	76.29	59	30	93	2
Bleomycin(μg)							
2	87.13	1.5	73.09	41	30	71	2
4	98.3	1.9	75.29	43	42	85	2
8	96	2.1	95.27	16	33	49	1
10	92.63	1.5	82.09	31	32	63	2
Doxorubicin (μg)							
10	108.22	17.9	78.17	40	29	97	2
20	87.91	30.4	79.55	55	47	139	3
25	91.07	60	30.01	37	13	66	2
30	81.94	45.5	86.25	35	14	75	2

was assessed by MTT assay and percentage of apoptotic cells was determined by flow cytometry (Annexin V/PI assay).

3. Results

3.1. Effect of drugs on cell viability and apoptosis

Upon treatment with different concentrations of diethylstilbestrol cell viability was found to decrease in a dose dependent manner whereas treatment with different concentrations of Bleomycin, Mitomycin C and Doxorubicin led to mild reduction in cell viability at some of the concentrations used. No reduction in cell viability was however observed upon treatment of HepG2 cells with Griseofulvin and Vincristine (Table 1).

Similarly, the cells treated with Diethylstilbestrol, Doxorubicin and Vincristine demonstrated an increase in percentage of apoptotic cells at different concentrations used. Cells treated with Mitomycin C, Bleomycin and Griseofulvin showed a variable effect with presence of few apoptotic cells at certain concentrations (Table 1).

3.2. Effect of drugs on numerical CIN (Aneuploidy)

The untreated HepG2 cells showed a $42.77 \pm 4.20\%$ population of aneuploid cells. An increase in aneuploid population was observed at all or most of the drug concentrations upon treatment of HepG2 cells with diethylstilbestrol, Mitomycin C, Bleomycin and Doxorubicin. With Griseofulvin maximum aneuploid population (67.35%) was observed at 20 μg dose whereas with Vincristine no increase in aneuploidy was observed at any of the drug concentrations used (Table 1).

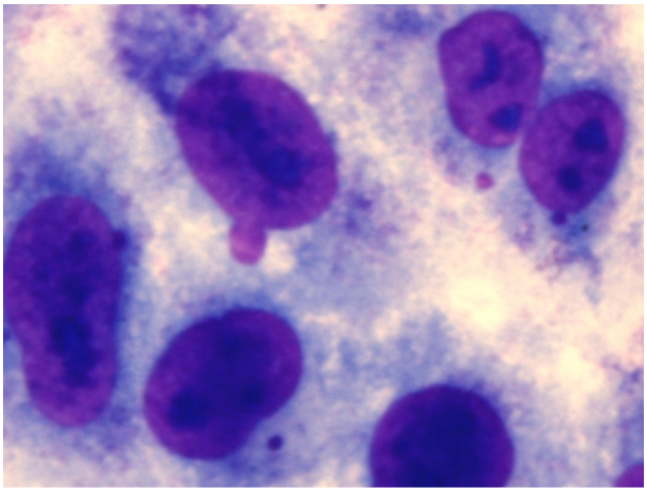

Fig. 1. Microphotograph showing most commonly observed morphological indicators of CIN like MN (arrows) and NB (arrowhead).

3.3. Effect of drug treatment on morphological CIN score

Amongst the above indicators studied MN were most frequently observed followed by NB (Fig. 1). Other parameters like NPB, CS, La and MPM were not common. Treatment with Diethylstilbestrol and Vincristine did not show any increase in morphological CIN score (Untreated = 1, Treated = 1) however, upon treatment with Griseofulvin, Bleomycin and Doxorubicin an increase in CIN score was observed at most of the concentrations used (Control = 1, Treated = 2/3). A maximum CIN score of 3 was noted in case of Doxorubicin at 20 μg dose in HepG2 cells.