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Radiation sensitivity and sensitization in melanoma

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Dear Editor,

Locally advanced melanoma is clinically challenging; melanomas are considered radioresistant (Harwood & Cummings, 1981). Recent reports, however, show variable radiosensitivity in patients and established cell lines (Stevens & McKay, 2006) (Sambade et al, 2011). Our purpose was to confirm this in early passage, patient-derived melanoma cultures and study radiosensitizing potential of commonly used melanoma drugs: temozolomide, carboplatin, paclitaxel and vemurafenib.

We studied 14 cultures with/without BRAF/NRAS mutations and PTEN loss. Seven efficiently formed colonies *in vitro*. We determined that the less cumbersome, luminescence-based CellTiter-Glo assay (Promega) was a good surrogate for colony forming assays. Radiation inhibitory doses 50 (ID_{50}) from colony-forming and CellTiter-Glo assays correlated well (R=0.8) (sFigure 1). ID_{50}s by CellTiter-Glo were established using ascending doses (2Gy, 5Gy, 8Gy), representing common conventional fractionation and hypofractionation doses (Table 1). We detected a range of radiation sensitivities (Figure 1); YUROL, YUTICA and YUMUT were 45-60% viable with 8Gy; growth was <20% in YUSIT1, YUGASP, and YUSAC cells. YUROL and YUSAC differed in radiosensitivity by >7-fold at 8Gy. All were relatively radioresistant at low ionizing radiation (IR) doses. The surviving fraction at 2Gy (SF2) is a common measure of intrinsic radiosensitivity; SF2≥0.5 is considered radioresistant (Fertil & Malaise, 1981). Our SF2 range was 0.52-0.88, mean-0.73±0.088. Although the number of cultures is small, when dichotomizing ID_{50}s by their median, PTEN loss was associated with high ID_{50}s (χ^2=5, *P*=0.026). No association was found between BRAF/NRAS mutations and ID_{50}.

For radiation sensitization, we determined inhibitory concentration 50 (IC_{50}) for individual drugs (Table 1). Cells were treated with concentrations at or below the average IC_{50} and irradiated 4 hours later. Combination indices (CIs) were calculated using CalcuSyn software, incorporating the Chou and Talalay method; CI <0.9 is synergistic, 0.9-1.1 additive and >1.1 antagonistic.

All cultures but one were resistant to temozolomide. We focused our analysis on 2Gy and 5Gy, conventional irradiation doses. Temozolamide at 100μM was synergistic with 2Gy in six cultures and with 5Gy in four, although growth remained >50% in many (sTable 1, Figure 2A). Temozolomide induces guanine methylation, repaired by O6-methylguanine-DNA methyltransferase, which can be hypermethylated in melanoma (Carlson et al, 2009). It is unclear that this synergizes with IR-induced double-strand breaks (DSBs) (Wedge et al, 1997).

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IC_{50}s for carboplatin were 4.6-57.6μM. There was some synergism between 2Gy and 3μM carboplatin in two cultures, but not with 30μM. Synergistic effects were more abundant in carboplatin-treated cultures (30μM) with 5Gy; seven of 14 showed synergism and five additivity, with <50% growth (sTable 2, Figure 2B). Although cultures are fairly sensitive to 30μM carboplatin, adding radiation clearly enhances growth inhibition. Carboplatin creates inter- and intra-strand adducts, synergizes with IR-induced DNA damage to create DSBs (Yang et al, 1995). Additional mechanisms include non-homologus end-joining repair inhibition, hypoxia, and cell-cycle dysregulation.

IC_{50}s for paclitaxel were 1-62nM (3 day exposure). 3nM and 2Gy were synergistic in eight cultures, additive in five (sTable 3, Figure 2C). 10nM was synergistic with 5Gy in two cultures, suggesting that paclitaxel radiosensitizes primarily at lower radiation doses. Microtubule stabilizing agents block cells in the radiosensitive G2/M cell division phase (Liebmann et al, 1994).

IC_{50}s for vemurafenib in mutated BRAF cultures were 18-160nM, lower than in repeatedly passaged, older cultures used by Sambade et al, but similar to those in our early passage cultures in prior studies (Halaban et al, 2010; Sambade et al, 2011). 100nM vemurafanib was synergistic with 5Gy in six cultures (sTable 4, Fig 2D), confirming other studies (Sambade et al, 2011).

Our data demonstrate that radiation sensitivity is variable in short term melanoma cultures, which may be better surrogates for *in vivo* response patterns than established cultures, many being radio-sensitive, particularly at higher doses. This supports previous reports that melanomas have low alpha-beta ratios, predicting enhanced sensitivity to hypofractionation (Overgaard et al, 1986). PTEN loss might be associated with radioresistance, as demonstrated in gliomas (Inaba et al, 2011). Carboplatin and paclitaxel, used as radiosensitzers in other diseases (Groen et al, 2004), and vemurafenib appear to be more effective than temozolomide. The greatest growth inhibition was seen with higher carboplatin concentrations and 5Gy; this combination might be appropriate in anatomic sites that tolerate higher doses of radiation. Paclitaxel and carboplatin can be administered in single, high doses, and pre-treating patients with one or both prior to irradiation might improve local tumor control. *In vivo* studies are needed to assess toxicity and optimal dosing schedules of newer drugs such as vemurafenib, when used together with irradiation. Studies are underway to identify additional biomarkers (other than PTEN loss) that predict radiosensitivity in melanomas.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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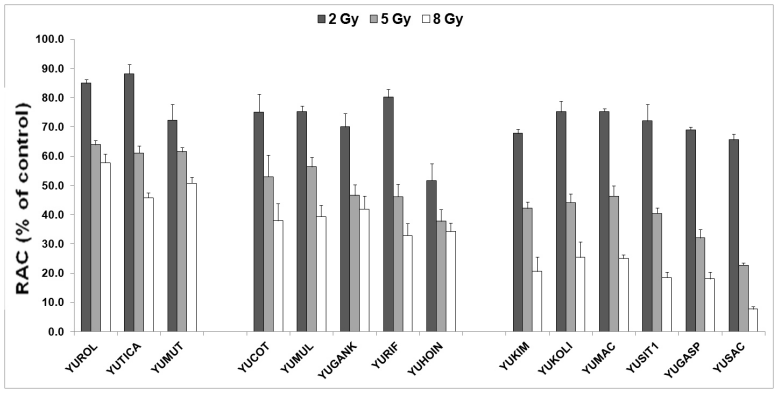

Figure 1.
Sensitivity of primary melanoma cultures to increasing doses of x-ray irradiation. The Y axis depicts the relative ATP content (RAC).

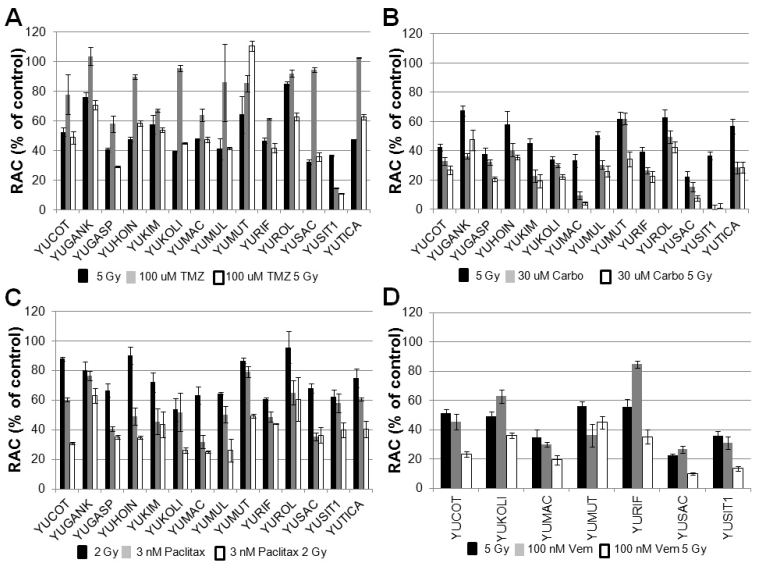

Figure 2.

Combined effects of cytotoxic drugs and x-ray irradiation. The more effective combinations are shown for the four drugs studied; temozolomide (A), carboplatin (B), paclitaxel (C) and vemurafenib (D). Standard errors were calculated based on three independent experiments, each done in triplicate. The Y axis depicts the relative ATP content (RAC).

Table 1

Radiation inhibitory doses 50 (ID_{50}) and inhibitory concentrations 50 (IC_{50}) for cytotoxic drugs used to treat melanoma in clinic.

Cell culture	BRAF	NRAS	PTEN	irradiation	TMZ	Carbo	Paclitax	vemurafenib
	Mutation	mutation	expression	ID_{50} (Gy)	IC_{50} (μM)	IC_{50} (μM)	IC_{50} (nM)	IC_{50} (nM)
YUCOT	V600E	WT	+	5.4	NR*	26.7	4.3	94.0
YUGANK	WT	Q61K	+	5.0	NR	57.6	4.3	NT**
YUGASP	WT	Q61L	+	3.2	100	12.7	4.1	NT
YUHOIN	WT	WT	+	2.2	NR	33.1	4.0	NT
YUKIM	WT	Q61R/WT	+	3.6	NR	3.1	1.0	NT
YUKOLI	V600E/WT	WT	+	4.2	NR	14.4	2.9	138.0
YUMAC	V600K	WT	+	4.3	70	4.6	2.4	18.0
YUMUL	WT	WT	−	5.8	NR	11.3	2.5	NT
YUMUT	V600E/WT	WT	−	8.0	NR	46.1	1.3	160.0
YURIF	V600K	WT	+	4.8	NR	5.0	3.8	132.0
YUROL	WT	WT	−	NR>8	NR	27.9	61.7	NT
YUSAC	V600E	WT	+	2.7	NR	7.0	4.9	22.0
YUSIT1	V600K/WT	WT	+	3.7	15	6.0	4.1	21.0
YUTICA	WT	Q61R/WT	+	7.0	NR	10.3	4.2	NT

NR-Not reached, NT-Not tested, temozolomide is denoted by TMZ, carboplatin by Carbo, paclitaxel by Paclitax,