

Single dose irradiation of GH₃ cells increase GH and PRL secretion *in vitro*



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GH-secreting pituitary tumors may require multimodal therapy, including surgery, drugs- in particular somatostatin analogs (SSA) - and radiotherapy (RT). The aim of treatment is to normalize the hypersecretion of GH/IGF-1 responsible for acromegaly and to control tumor size. RT is indicated in a subset of patients with persistent disease after surgery or during medical therapy, allowing to control tumor growth in 85-95% of cases and to obtain a slow reduction of hormonal hypersecretion (up to 10-20 years after treatment). Some authors suggested interruption of SSA treatment during RT period because lowering the mitotic activity may reduce the efficacy of RT. However, the short term effects of RT on GH-secreting tumors have been poorly studied. Therefore, we aimed to evaluate effects of radiotherapy on hormone secretion in the rat GH/PRL-secreting cell line (GH₃) *in vitro*.

MATERIAL AND METHODS

GH₃ cells were plated 48h before irradiation with 5 and 10Gy dose using LINAC ELEKTA Synergy (Ospedale S. Salvatore, L'Aquila) (Fig. 1). The effects of treatment were evaluated both early (6 and 24h) and late time points (72 and 144h). Cells were counted with a Burkner chamber and vitality was assessed using Trypan blu 0.5% exclusion. **ELISA:** GH and PRL secretion were determined in culture media by specific enzyme-linked immunoassays (SPL-BIO, Bertin Pharma, France) and corrected for cell number. All experiments were performed in duplicate and repeated at least twice. **qRT-PCR analysis:** Total RNA was extracted from collected cells using Eurogold TriFast (EuroClone, Pero, Milan); cDNA was obtained from 1µg of RNA and semi-quantitative Real-time RT-PCR (sqRT-PCR) of cDNA was performed in duplex for each sample using commercial Taqman assays (Life Technologies, Monza) for rat GH and PRL with Cyclophilin B as housekeeping gene.

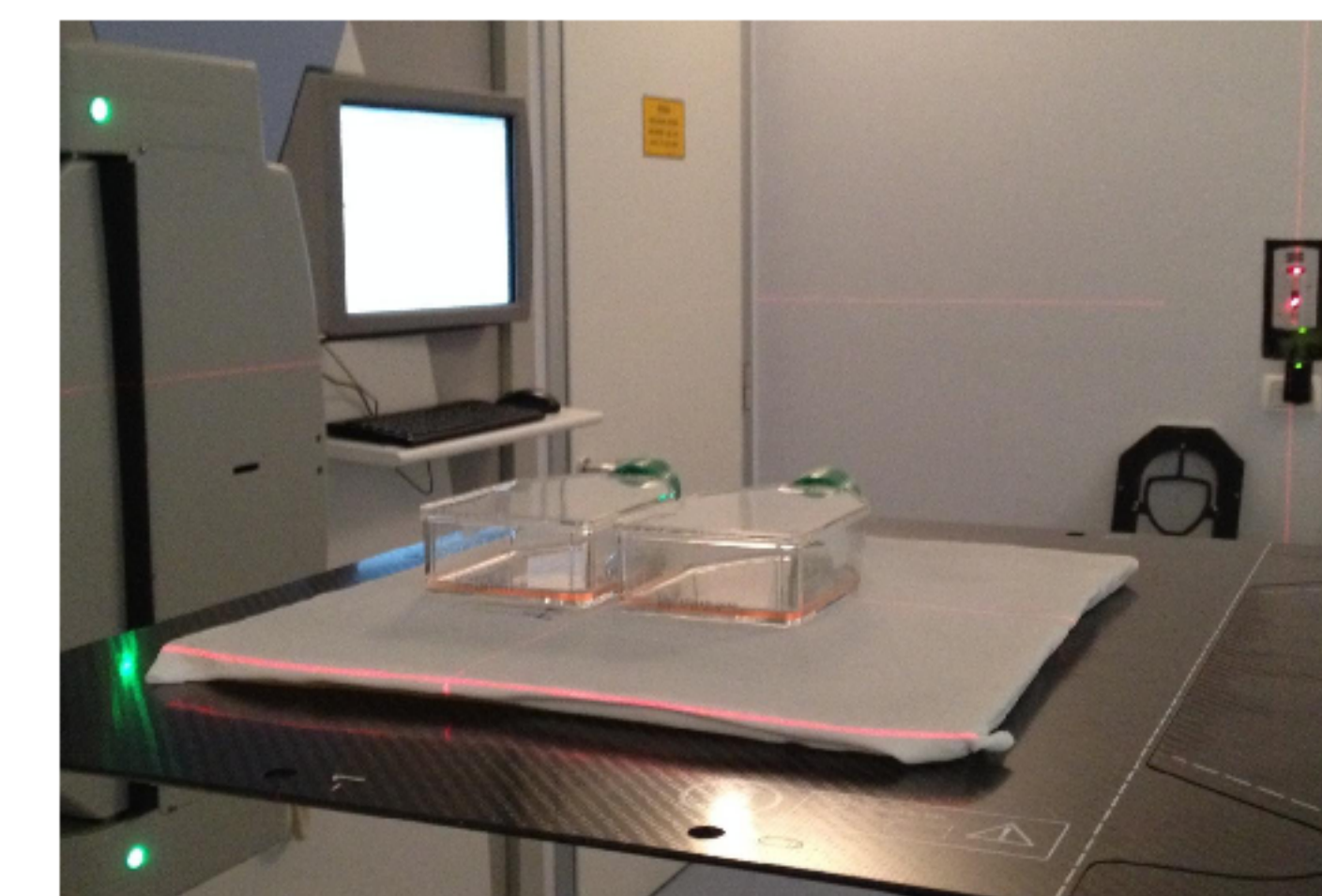


Fig. 1 RT treatment



RESULTS

(1) GH₃ cell growth after irradiation

Radiotherapy has induced a time and dose-dependent reduction of cell growth compared to control cells (Ct) (Fig. 2), which became significant at 24h of treatment at high dose (P=0.001 10 Gy vs Ct), and more pronounced at later time points (P<0.0001 vs Ct at 72 and 144h for both treatment doses, P=0.004 10Gy vs 5Gy at 144h). A marked reduction in cell viability was observed at late time points (up to 90 and 80% after 5 and 10 Gy at 144h, respectively). **NB:** in Fig 2b dark blu are for additional control cells transported to the radiotherapy unit without treatment.

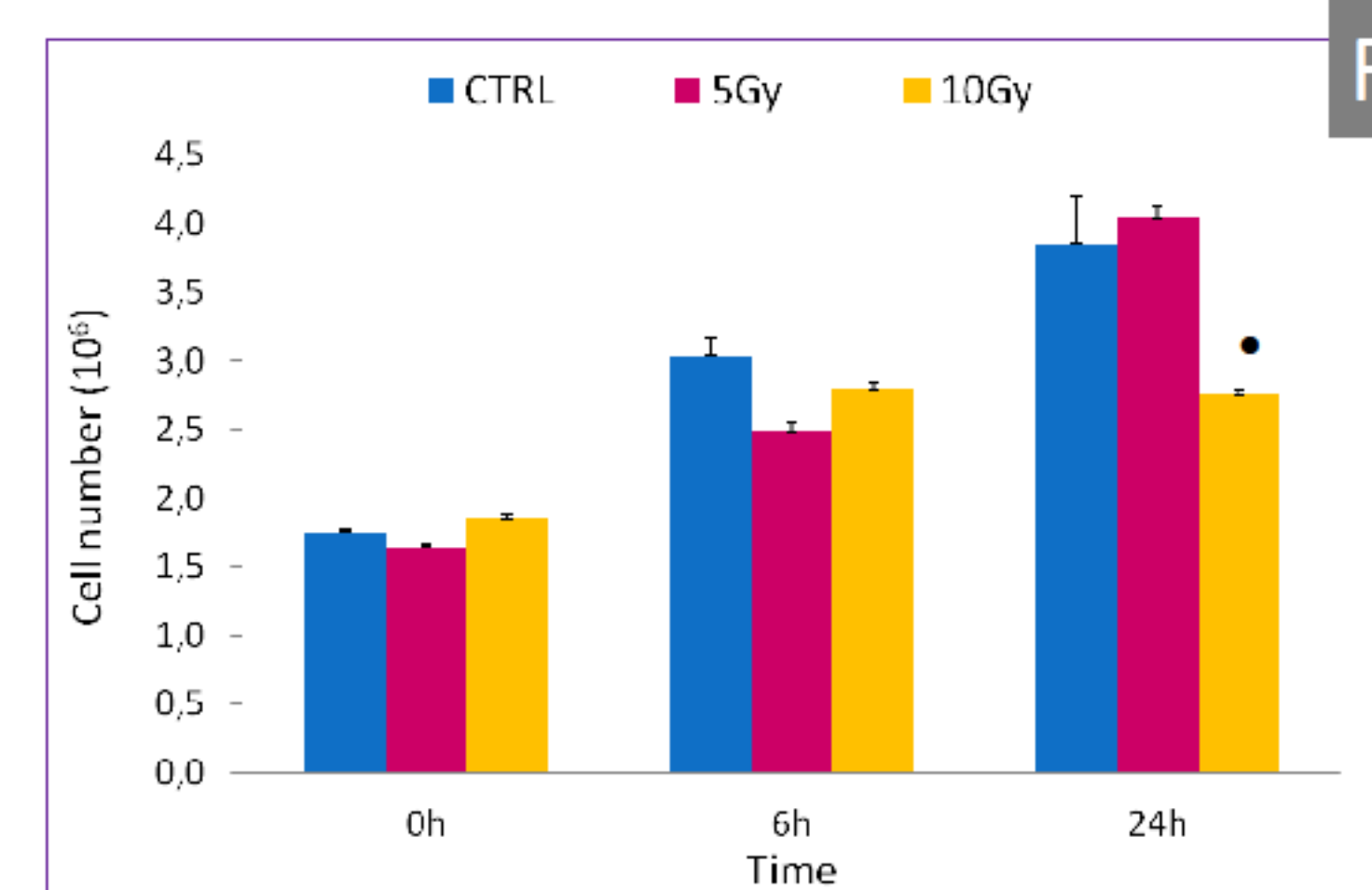


Fig 2a: 6-24 h

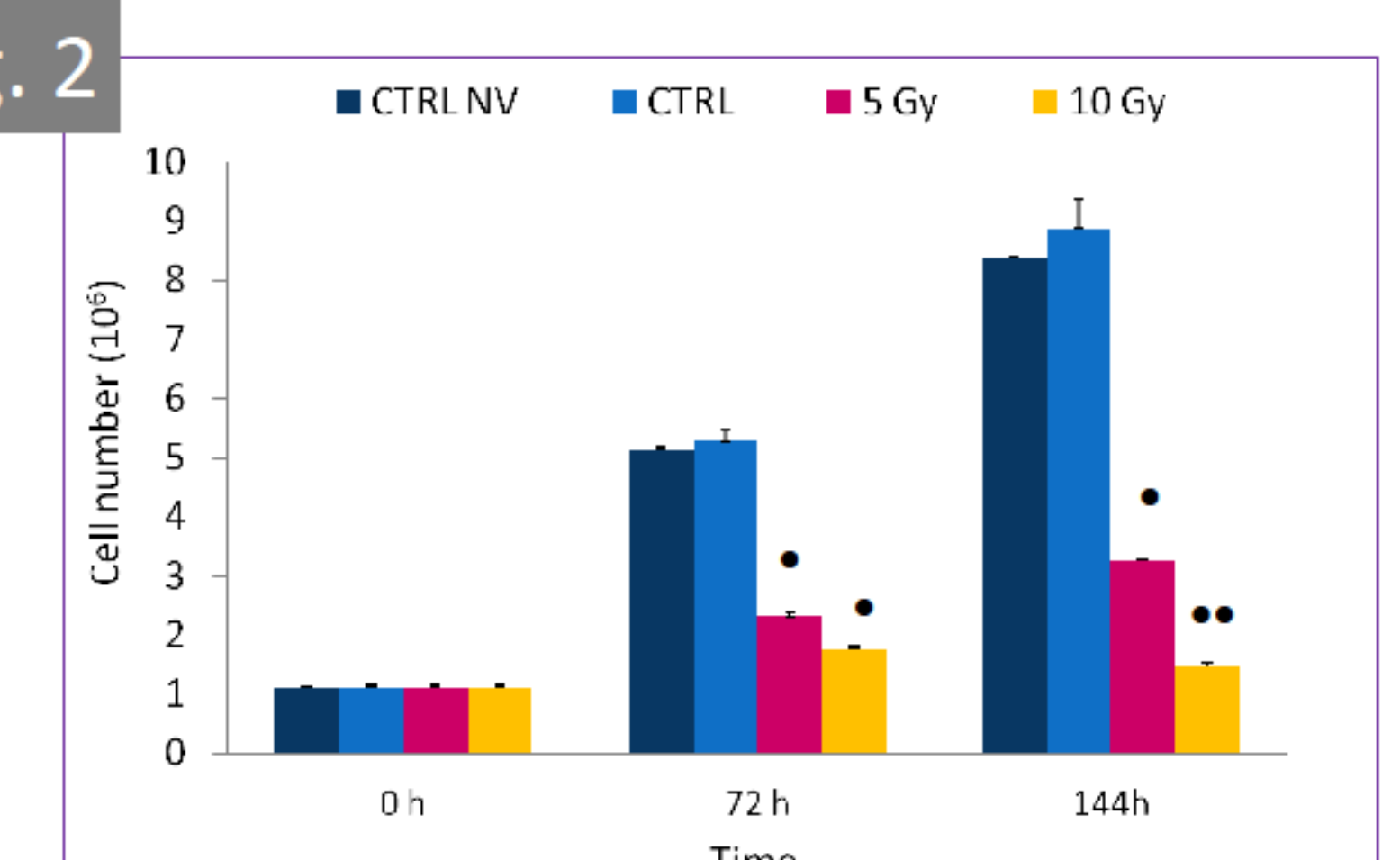


Fig 2b: 72-144h

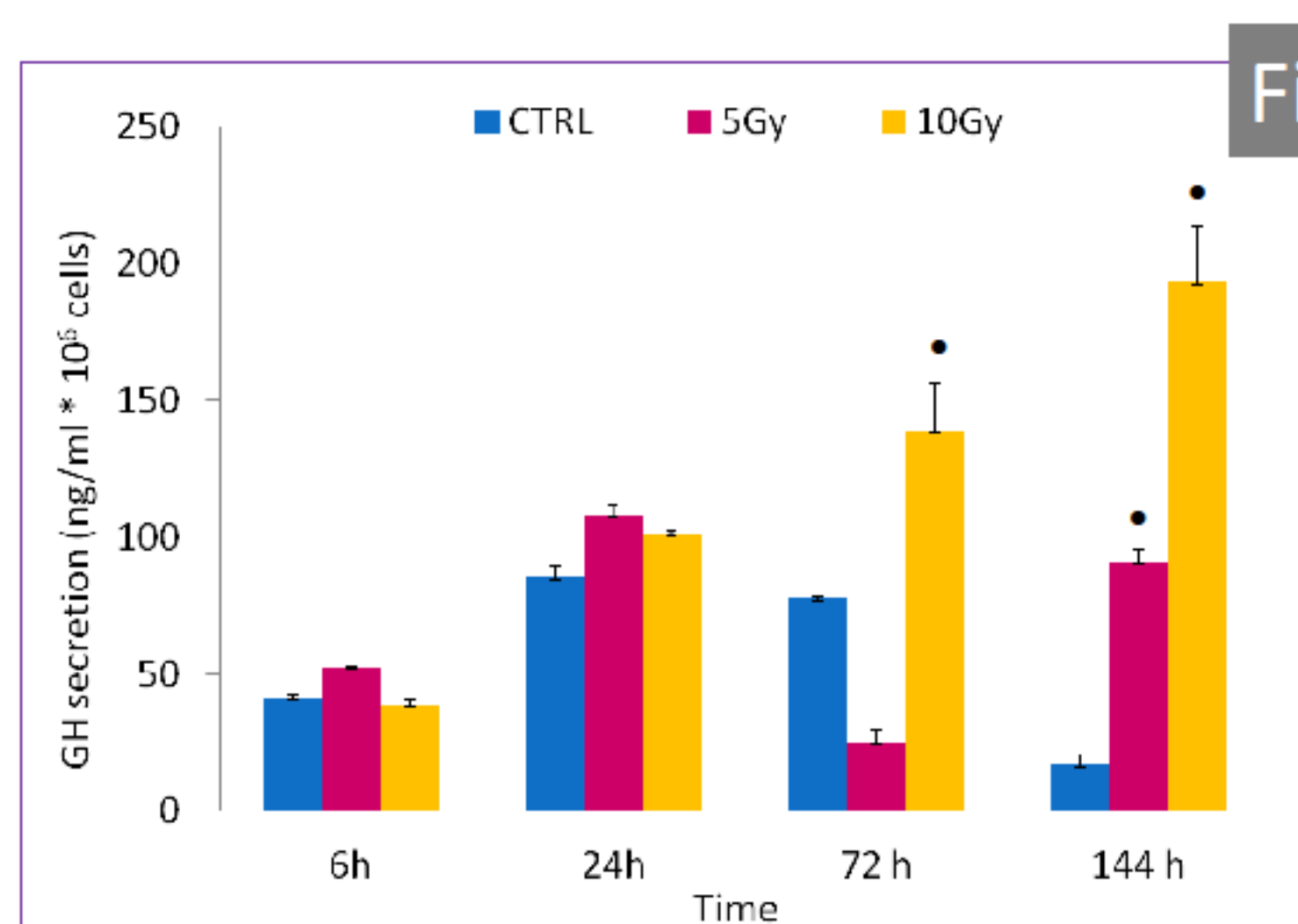


Fig.3a: GH secretion

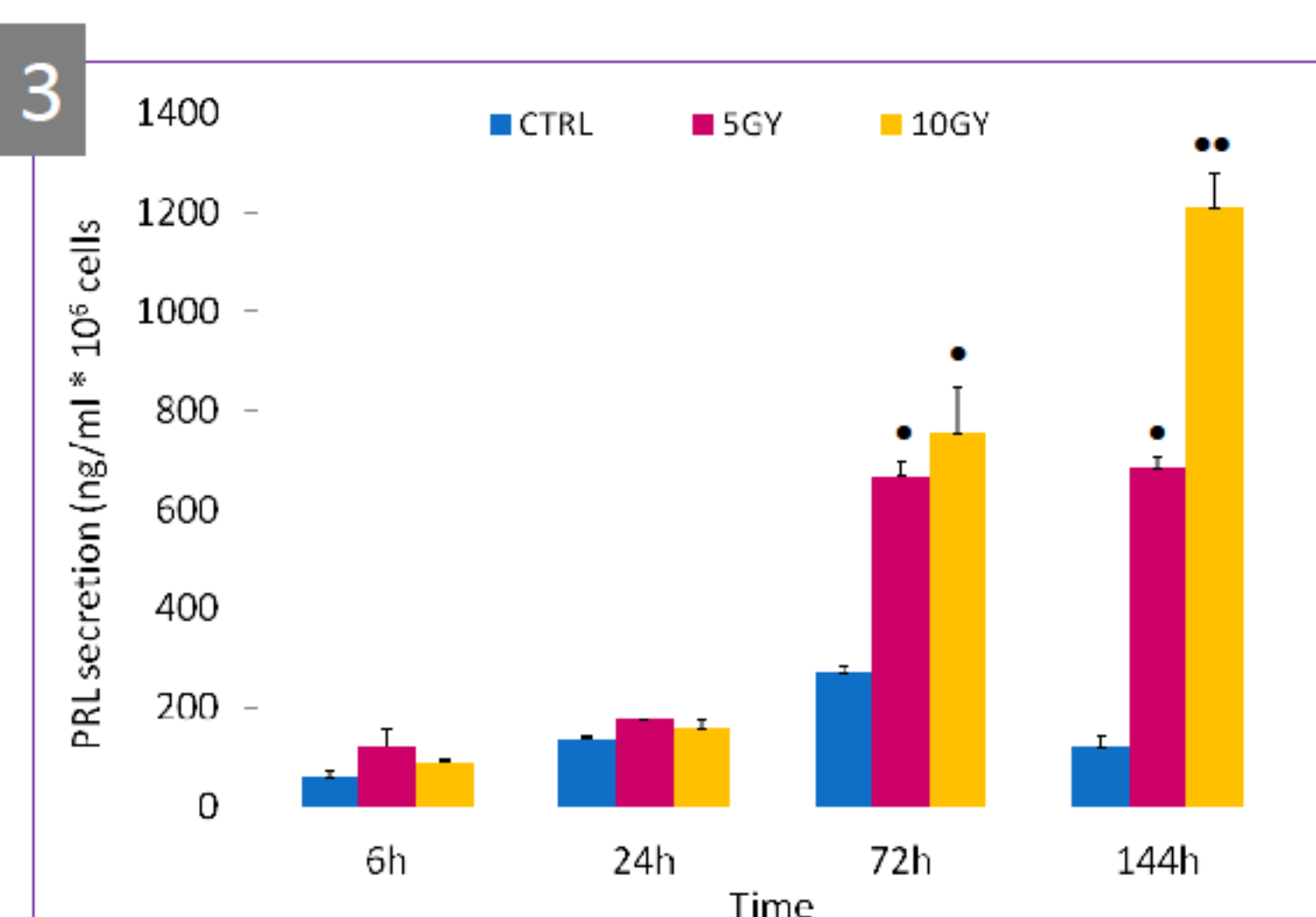


Fig.3a: PRL secretion

(2) Radioterapy effect on GH and PRL secretion *in vitro*

In contrast, a progressive increase in GH and PRL secretion was observed, which was in contrast with the spontaneous reduction in control cells at late time points (Fig. 3). The effect on hormone release was dose-dependent, reaching nearly 5-folds when compared to control cells at 144h after a 10Gy irradiation (P=0.007 and P<0.0001 vs Ct at 144h for 5Gy and 10Gy, respectively). An even more pronounced increase in PRL secretion was observed - reaching up to 11 folds at late time points (P<0.0001 vs Ct after 144h for either dose).

(3) Effect of irradiation on GH and PRL gene transcription

In order to determine the mechanisms of hypersecretion of GH and PRL in irradiated cells, the transcription of both genes was evaluated in the same experiments. Interestingly (Fig. 4) we observed that cell irradiation had a differential effect on the transcription of GH and PRL genes. GH transcription was significantly increased at 24h with 10 Gy dose (P=0.007 vs Ct) and reduced at later time points (P<0.0001 at 72h and 144h vs Ct after 5 and 10Gy), suggesting a predominant effect on hormone release. In contrast, PRL transcription was found to increase at late time points both in control and irradiated cells (P<0.0001 at 144h vs 72h in all conditions).

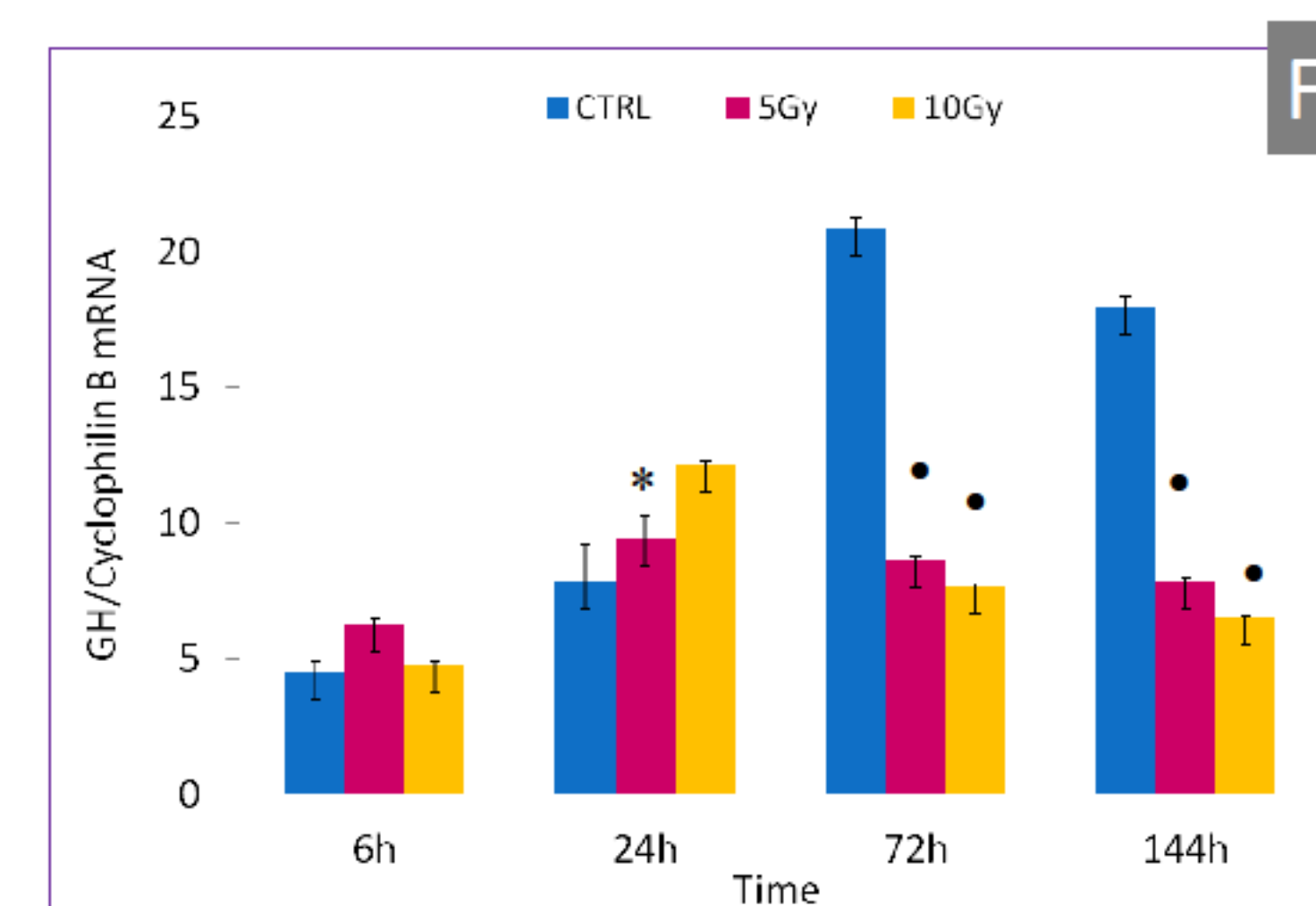


Fig.4a: GH mRNA

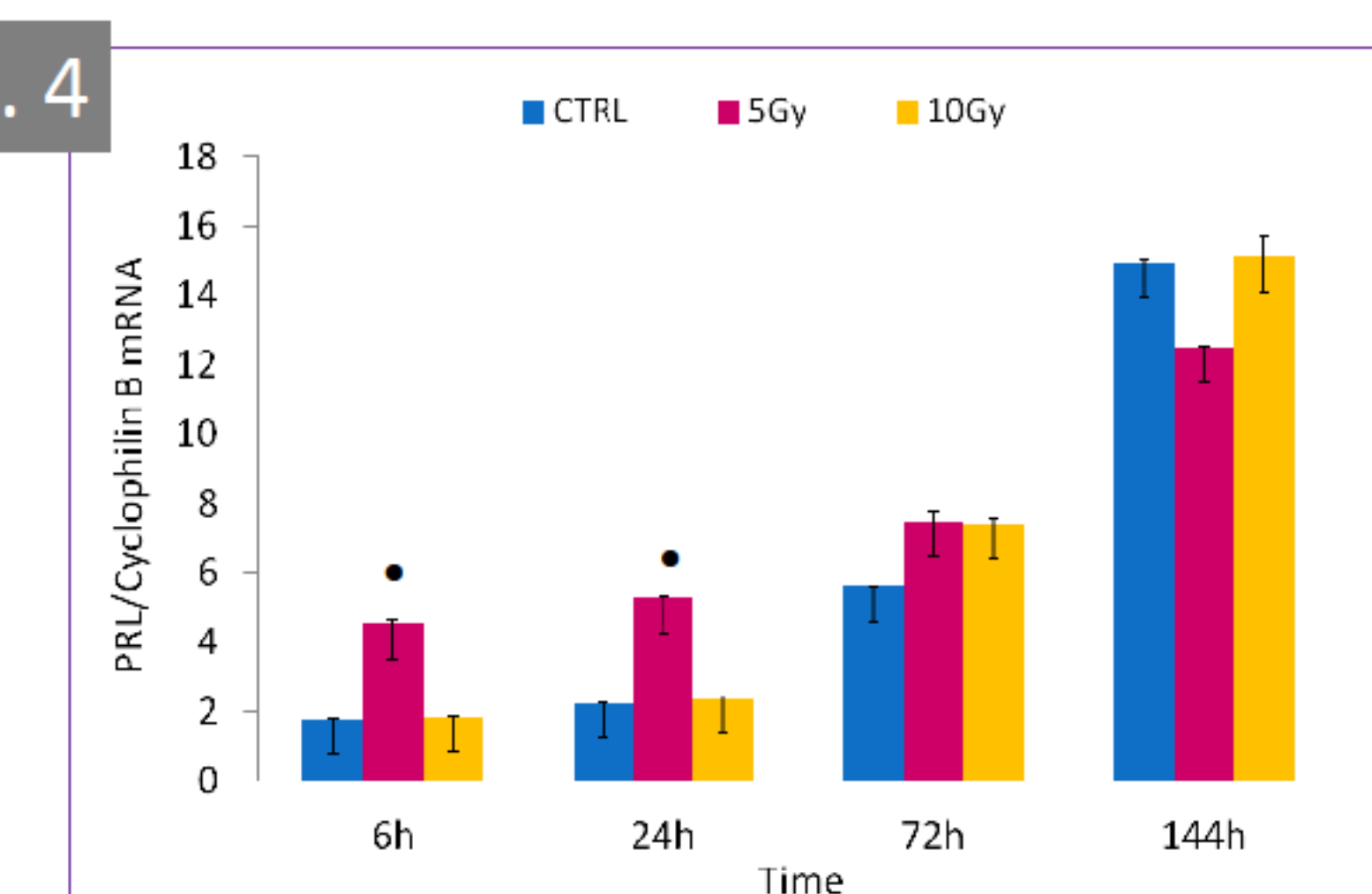


Fig.4a: PRL mRNA

CONCLUSIONS: A transient increase in hormone secretion may occur after irradiation of GH/PRL cells, which is likely to occur at least in part as a result of cell death, although these data suggest a more complex picture with differential effects of acute irradiation on GH and PRL synthesis and release. Further experiments are ongoing to determine the impact of SSA treatment *in vitro* on such parameters. We suggest that potential exacerbation of GH/IGF1/PRL hypersecretion secretion should also be evaluated *in vivo* in order to evaluate the effects of SSA withdrawal before irradiation in acromegalic patients

References: Mareková M & al Endocr J 2003, 50:621-8; Zeng J & al Cancer Biol Ther 2009, 12:657-663; Rezáčová M & al Physiol Res 2008, 57:225-35.



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