# Cardiac differentiation of human embryonic stem cells as a measure for radiation risk in early embryogenesis*

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The early embryo is particularly vulnerable to ionizing radiation responding to exposure with prenatal death, organ malformation or other detrimental ramifications [1]. Thus, a thorough assessment of the radiation risk is mandatory in situations of inevitable or unintended exposure of the conceptus *in utero*. However, our current understanding of radiation induced effects is predominantly based on animal studies or radiation accidents [2] hampering an in-depth evaluation of the underlying mechanisms. Human embryonic stem (hES) cells, derived from the inner cell mass of the blastocyst during embryogenesis, present a valuable tool to examine radiation effects on the early embryo as they can differentiate into all cells of the body mimicking human development *in vitro* [3]. Of special interest is the impact of diagnostic or therapeutic irradiation on the formation of the heart as one of the earliest organogenic processes taking place from week 2-7 in humans (for concise review see [4]). We established a hES cell-based cardiac differentiation protocol, which modulates the Wnt-signaling pathway that is crucial for *in vivo* cardiogenesis [5]. In an insulin-free culture it leads to the stepwise generation of mesoderm (by inducing Wnt via CHIR99021), cardiac mesoderm (by inhibiting Wnt via IWP2), cardiovascular progenitors and beating cardiomyocytes recapitulating all stages of the developing heart within 15 days (Figure 1). Using quantitative PCR, specific markers could be detected at every differentiation stage. In contrast to hES cells and spontaneously differentiating cells, in hES cells subjected to directed differentiation according to Lian et al. [5] the appearance of early cardiomyocytes (d8-d15) was marked by beating clusters and the expression of early cardiomyocyte markers such as alpha myosin heavy chain (aMHC, Figure 2). Further culture led to the synchronization of cardiomyocyte clusters with increased beating rates and an elevated expression of genes specific for mature cardiomyocytes. Successful cardiac development was also evidenced post-transcriptionally by increased levels of cardiac microRNAs. Currently, techniques to determine the beat-rate and other electrophysiological parameters in cardiomyocytes derived from non-irradiated or irradiated hES cells are established. Preliminary data suggest that the cardiac differentiation of hES cells surviving the exposure to 1 Gy X-rays is accelerated leading to premature expression of cardiac markers when compared to unexposed cells. Such acceleration may render the cells unsusceptible to subsequent positional and temporal patterning explaining radiation induced effects such as the above mentioned organ malformations or prenatal death. Currently, the underlying mechanisms are studied in more detail.

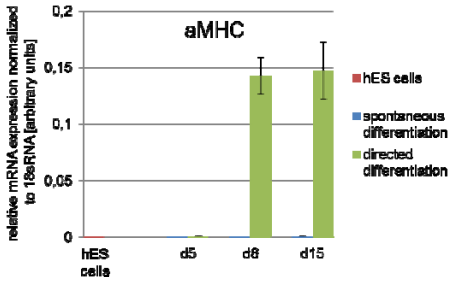


Figure 2. aMHC mRNA expression in cells subjected to spontaneous or directed differentiation according to Lian et al., d: days.

## References

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[5] X. Lian et al., Nat Protoc 8 (2013) 162.

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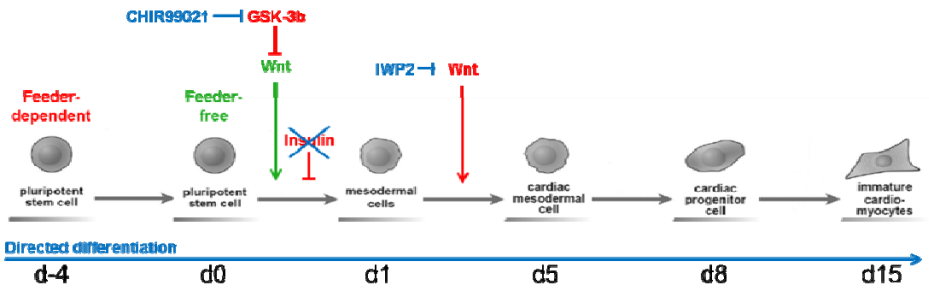


Figure 1. Differentiation scheme according to Lian et al. Differentiation promoting factors are depicted in blue and green, inhibiting factors are shown in red, d: day.