Alterations of the oncogenic properties of tumor cell lines by modulating oncogene expression. Example of v-*src* transformed chicken cell lines

Abstract

v-Src protein tyrosine kinase is the product of the transforming gene transduced by avian Rous sarcoma virus. In contrast to proto-oncogene c-*src*, v-*src* lacks the negative-regulatory C-terminal domain and consequently shows a higher level of activity and transforming ability. In addition, v-Src contains point mutations throughout its coding region that probably contribute to the high level of intrinsic kinase activity. Long terminal repeats (LTR) comprise strong promoter-enhancer sequences and ensure efficient expression of the v-*src* gene. v-Src protein has a strong transforming potential *in vitro* and induces tumor development and growth *in vivo*. Moreover, it is implicated in metastatic formation. In several cancer types the elevated c-Src kinase activity caused pleiotropic cellular responses inducing transformation and metastasis.

The aim of this diploma work was to reveal the role of v-Src in mediating tumor and metastatic progression in chicken cell lines PR9692 and PR9692-E9. Despite the low propensity of the chicken cells to immortalization, comparatively high immoratlization efficiency was observed in cells from *ex vivo* tumours growing progressively in chickens of the inbred line CC.R1. Among them are these two cell lines that originate from tumors induced in chickens of the inbred line CC.R1 by employing the moleculary cloned LTR, v-*src*, LTR proviral DNA. PR9692-E9 is a subclone of PR9692 derived *in vitro* by limiting dilution. Both cell lines overexpress transforming protein v-Src, but they differ in metastatic properties. Line PR9692 induces metastatic progression after inoculation in chicks whereas line PR9692-E9 does not. To study the role of v-Src we used short interfering RNA to silence v-*src* expression in the process that is known as RNA interference. We have prepared cell lines PR9692 and PR9692-E9 that stably express shRNA mediating knock-down of v-*src* expression. Knock-down is provided by plasmid-based gene silencing. We employed three different shRNA constructs that targeted v-*src*. The knockdown effect slightly varried among different constructs. The most significant reduction in v-*src* expression was acquired by shRNA against the v-*src* coding region compared with shRNA against the leader sequence or U3 region of v-*src*. The expression of v-*src* mRNA and protein were analyzed by RT-PCR and Western blot.

PR9692 and PR9692-E9 cells with reduced expression of v-*src* were inoculated into chickens to reveal whether the level of v-Src in these cells correlates with tumor induction and metastatic potential. We demonstrated that v-*src* is one of the genes playing an essential role in maintaining malignant phenotypes. In most cases inhibition of v-*src* in cell line PR9692 decreased the incidence of metastases, moreover the growth of primary tumors declined in all cases.

We conclude that the level of v-Src influences the primary tumor growth and the metastatic potential.